The Journal of HEREDITY

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Gamma Irradiation of *Lilium*

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Paroxysm—A Sex-Linked Lethal of Fowl

Temperature-Sensitive Alleles in Maize

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A STATE OF TETANY

Figure 1

Seven-week-old pullets showing the symptoms of paroxysm. The legs and digits are fully extended and rigid while the head is forced over the back.

PAROXYSM—A SEX-LINKED LETHAL OF THE FOWL

With a Note on the *xl* Lethal

R. K. COLE*

THE domestic fowl is blessed by a set of circumstances that favor the early recognition of sex-linked characters. The female is not only heterogametic and produced in large numbers for economic reasons, but a male will sire numerous progeny from a number of different dams. Thus, pedigreed populations of sufficient size, and in which the segregation of sex-linked characters can occur, are frequently produced. There have been no less than 15 sex-linked genes reported for the fowl[6], although the sex chromosome is only the fifth largest. The fifteenth sex-linked gene, *px* (paroxysm)[2], and its locus on the chromosome are described in this paper.

Four of these genes (*xl, sh, j, px*) have proven to be lethal to the hemizygous female, and it is interesting to note that in three and possibly all four cases there appears to be a disturbance of the nervous system. Furthermore, the mutant genes are or appear to be located in a region of the chromosome which is some distance from the locus for barring, possibly on the alternate arm beyond the spindle attachment region.

Among the female chicks from a crossbred sire (Houdan × White Leghorn), backcrossed to Anconas, there appeared a number which showed a set of symptoms with subsequent death that was unlike anything seen previously. Affected chicks were the progeny of only one of four similar sires and were produced by all of the 16 hens to which he had been mated. The genetic nature of the character was thus clearly established by this initial cross, for approximately one-half of the daughters of the one sire were affected. Subsequent studies on the linkage relationships with other sex-linked genes confirmed the mode of inheritance of the character as a sex-linked recessive obligate lethal.

The Lethal Syndrome

The pullet chicks which receive the gene *px* from their sire hatch normally and show no disturbance of behavior until about two weeks of age or older. At that time, following sudden auditory or visual stimulation, an occasional chick will start to move quickly across the floor of the pen or battery, as if excited, and then fall, with legs rigidly extended, head thrown back, wings beating violently, while the entire body is in a state of tetany and tremors (Figure 1). The tetany and tremors continue for about 10 seconds, after which the entire body becomes completely relaxed, the eyes are closed, and the chick lies quietly. After an interval of varying duration, depending upon external stimuli, the chick will get to its feet and stagger off. In so doing it appears to walk on its toes, and, in an attempt to overcome a tendency to fall forward, takes a series of short quick steps while holding its tail spread, like that of a strutting turkey.

The complete prostration of the affected chick during the immediate post-paroxysmal period is best illustrated by the following observation. Several chicks have actually drowned when their heads came to lie in the shallow water trough at the end of a paroxysm. They apparently lacked the strength or awareness of the need to lift their heads from the shallow water, only 1½ cm. deep.

Subsequent attacks occur at irregular intervals. With increasing age more of the *px* birds will show the complete syndrome, although not all of them will do

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so at the same time. Any disturbance which surprises the bird may bring on an attack. Noise, bright light, movement, or merely picking up the chick can precipitate the syndrome. Once the birds are aware that someone is present these stimuli are much less effective in precipitating the syndrome, indicating, perhaps, that some centralized control can be exerted to regulate the initiation of the reflex mechanism. Furthermore, once the birds have been through a tetanic spasm they require a period of half an hour or longer for recuperation before another attack can be induced. With time, and after a series of attacks, the *px* birds can be recognized by the constantly spread tail, the unsteady gait, and poor growth. Eventually all of them succumb, usually by 14 to 15 weeks of age. No pathological changes in gross structure have been recognized nor have any microscopic lesions been seen in sections from the cerebellum.

It has been observed that the syndrome will be expressed when the *px* birds are killed by dislocation of the neck (stretched to sever the spinal cord in the anterior cervical region). Of 37 *px* chicks, previously observed to have shown the complete syndrome, 32 did so again when the neck was dislocated. Among 12 which were recorded as having shown the syndrome, but not actually observed to do so by the author, 11 did so when killed. Another group of 14 which showed the symptoms of poor growth, stilted gait, and spread tail, but which had not been observed to exhibit a paroxysm, were classified as *px*. When these were destroyed, 11 exhibited a typical paroxysm. Of the nine birds (in the above groups) which did not express the syndrome when killed, four showed no post-mortem convulsive movements of skeletal muscles. Normal birds killed by this method will show considerable and violent movement of the limbs for a period of 10 to 20 seconds, but at no time will they exhibit the rigidity of the body and legs that is so characteristic of the known lethal birds. It is believed that these four birds, mentioned above, were in a post-paroxysmal period when killed and hence incapable of exhibiting the spasms. Among the other five birds, none of which could be distinguished from normal birds on the basis of their reaction when killed, there were two which had been observed previously to exhibit a typical paroxysm.

Linkage Studies

Independence of gene B

The original carrier male was heterozygous for barring, *B*, as well as for the lethal gene, *px*. Among his normal daughters surviving to 150 days of age, when classifications for various genes were first recorded, there were 22 classified as *b* and 18 as *B*. Other chicks from this mating carried dominant white, *I*, and hence classification for barring, in most cases, was not possible. These limited data indicated very loose linkage with *B*, in spite of the fact that *px* was also being transmitted to approximately half of this sire's daughters.

Among various male descendants, proven by test to be heterozygous for the lethal (*Px px*), there have been nine which also were heterozygous for the barring gene (*B b*) and among the progenies of which classification for

TABLE I. The linkage of *px* with other genes borne on the sex chromosome of the fowl

Sires		Female progeny, number				Gametes total	Crossing-over
		Parentals		Cross-overs			
No.	Genotype					No.	%
8	*B px / b Px*	88	102	114	98	402	52.7
3	*Al px / al Px*	119	121	17	11	268	10.5
9	*N px / n Px*	155	(140)	10	(0)*	165	7.2
		18	19	4	1	42	
1	*K Px / k px*	76	53	8	9	146	11.6

*The absence of individuals in this class makes it desirable that the 140 birds in the corresponding class not be considered when calculating linkage.

these genes was possible. In each of the males the two genes were in the repulsion phase. The ratios among the 402 pullets classified for both genes were approximately equal for each of the four classes (Table I). There were 212, or 52.7 percent, showing cross-over combinations of the two genes. The ratios of the segregations for the individual alleles were 202:200 for barring and 216:186 for the lethal. These data, like those from the original cross, indicate independent assortment, in spite of the fact that both genes are borne on the sex chromosome.

Close linkage with gene al

A sire known to be heterozygous for *px* was mated to an albino (*al*) dam. Subsequent testing identified three sons which were carriers of the *px* gene. They were of necessity also heterozygous for *al* because their dam was an albino. These genes, to be tested for linkage, were therefore in the repulsion phase and linkage could be measured in the female progenies from these sires. There was 10.5 percent crossing-over among 268 gametes tested (Table I). The individual values for the three carrier sires were 9.0, 9.2, and 12.5 percent. The gene *al* does not affect the viability of the chick either before or after hatching. The ratio of *Al* to *al* in the chicks hatched was 141:143 and in the chicks used to measure linkage 136:132. In these data the ratio of *Px* to *px* was 138:130.

Closer linkage with n

Sires known to carry *px* were mated to naked (*n*) dams. Nine sons were proven by test to be heterozygous for *px*, and also, as expected, for the *n* gene received from the dam. The female offspring from the nine sires (*N px* / *n Px*) were classified at two weeks of age or later for these two genes (Table I). Among those so classified there was a deficiency of naked chicks, but this was due to the greater post-hatching mortality among them than among the feathered ones. Among all females hatched there were 215 naked to 197 feathered pullets. The semi-lethal nature of the *n* gene to the hemizygous female, especially during the early period of life, was expected to cause a greater loss among these pullets than among their feathered sisters[7]. Of the naked pullets hatched, 33 died at that time or before transfer to brooders, while another 40 died during the first few days of brooding. Two were unaccounted for, leaving a total of 140 which lived and for which the presence or absence of *px* could be established. Not a single naked chick in this series showed any indication of the lethal syndrome. Among the feathered pullets, eight were dead at hatching time, 21 died during the first few days of brooding, and one was unaccounted for. Of the 165 kept long enough to determine the presence of *px* all but 10 showed the lethal character.

The absence of individuals in one of the cross-over classes (*px n*) makes it necessary to calculate linkage from the classes that were feathered. Among 165 gametes tested, 10 were cross-overs, giving a percentage of 6.1. It is quite probable that among the 40 *n* chicks which died during the early period mortality fell more heavily upon the double recessive, *n px*.

In a small mating of three carrier sires to Rhode Island Red females, to obtain material for a demonstration, the female progeny obtained were classified as follows: 4 *N Px*, 18 *N px*, 19 *n Px*, and 1 *n px* (Table I). Only one feathered and one naked chick had died before classification for *px* was possible. Although these data are limited, they do show that in the absence of early mortality associated with the naked condition, both *n* and *px* can be expressed in the same individual.

When these two series of data are combined, and after the exclusion of the parental class of 140 naked pullets from the first series, *px* and *n* show a linkage relationship of 7.2 cross-over units among 207 gametes tested.

Linkage with k

The gene *px* was first encountered in a male that was homozygous for rapid

feathering (*k k*), and since he had been mated to rapid-feathering Anconas, none of his sons could be used to test linkage of *px* with *k*. The naked dams and the albino dam, mentioned above, proved to be *k*. Subsequent crossing to known slow-feathering (*K*) Rhode Island Red dams yielded only one son among 11 tested which was a carrier of *px*.

This male, heterozygous for *px* and *k* in the coupling phase, was mated to Barred Plymouth Rock hens and produced 146 female offspring which could be classified for both genes (Table I). The deficiency of the double recessive class (53 *k px*) could not be attributed to errors in classification, for the *K* and *k* females were easily distinguishable at 10 days of age and the post-hatching mortality was negligible. The two cross-over classes were equal, as expected.

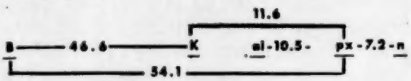
Because of the deficiency in one of the parental classes, these data are not the best for establishing the exact linkage relationship of *px* and *k*. However, if all the data are considered, there was a crossing-over between *px* and *k* of 11.6 percent.

In this cross, equivalent to a back-cross, the ratios of *K* to *k* and *Px* to *px* differ somewhat from the expected one to one. In the absence of a test of the linkage of these two genes in the repulsion phase, it is not possible to apply the method of Fisher[3] for estimating the recombination fraction in cases where there has been a disturbance in segregation of one or both factors. Since the data are limited, the linkage relationship calculated therefrom must be considered as an estimate of the true map distance.

Location of *px* on the Sex Chromosome

The well-known *B* and *K* genes, which are nearly 50 cross-over units apart, have been used in a three-point linkage test with *px* that yielded 146 pullet offspring. The gene *px* was found to be 11.6 cross-over units from *K* and independent (54.1) of *B*. In these same data *B* was 46.6 units from *K*. The double cross-over classes included only three chicks if *px* was assumed to be more distant than *K* from *B*. If *px* lies between these two loci, then there were 14 double cross-over gametes. Obviously the former arrangement is the correct one. From the tentative linkage of *px* with *n* and *al* reported earlier[2], Hutt[6] has assigned the *px* gene to its proper location on the sex chromosome. His data show a distance of 17.6 units between *n* and *al*. Since this figure is almost exactly the sum of the distance from *px* to *n* (7.2) and to *al* (10.5) now reported in this paper, it is clear that *px* lies between *n* and *al*, as Hutt placed it.

Data from the present study would place the genes on the sex chromosome as follows:



The data of Hutt[6] show that *al* is probably to the left of *K* by about 1.5 units. In the relatively limited data of the present studies a difference of two or three percent in crossing-over could be due to chance alone. Furthermore, the deficiency in one parental class, the double recessive, in the linkage test of *k* and *px*, is a little greater than that due to chance at the five percent level of probability. If this class and its associated one (*k Px*) are not included in the data for measuring linkage, then for the remaining data, in which one can have more confidence, the linkage relationship between *px* and *k* is found to be 9.5 units of crossing-over. Under these circumstances the linkage arrangement of *al - k - px - n*, as determined by the present data, is in complete and extremely close agreement with that derived by Hutt[6] from his extensive experiments.

Other Sex-Linked Characters

Three reports in the literature deal with sex-linked conditions affecting the nervous system of the fowl that were not cited by Hutt[6].

Kawahara[8] described a nervous disorder affecting pullet progeny from a mating of a cock to its half-sister. The character was lethal before maturity to approximately half of the afflicted pullets from this and other matings. Subsequent tests, including crossing of surviving affected dams to heterozygous

sires, produced affected males and females in proportions expected of a sex-linked gene. Except for differences in time of appearance of the tremors (earlier) and in specific Purkinje cells showing degenerative changes (gyri region of cerebellum) as well as survival of about half of the afflicted individuals, this character appears to be identical to shaker, for which the gene *sh* had already been established. Pending appropriate tests for allelism, one could assume that slight differences in expression of the gene action could be the result of modifying genes.

The non-lethal avian cerebellar hypoplasia described by Winterfield[11] was not definitely established as due to a sex-linked gene. Symptoms were first recognized at 12 weeks of age and in 12 different flocks, each of approximately 300 pullets. These came from one or two flocks of hens mated to New Hampshire males. Cockerel chicks were marketed before symptoms appeared in the sisters. If this cerebellar hypoplasia were due to a sex-linked gene, as suggested by Winterfield, some of the cockerels must have been heterozygous. To get a level of three to 10 percent incidence in the pullet progeny from mass matings would mean that approximately one in 10 of the sires must have been a carrier. At this level it would seem that the pedigreed New Hampshire flock from which they came must also have had other heterozygous sires, yet "no abnormal symptoms were reported in the parental stock."

Markson *et al.*[10] described a cerebellar degeneration and atrophy that appeared to be due to a sex-linked recessive gene. As in the condition described by Winterfield[11], it was not lethal but did markedly reduce reproduction. Although their data were very limited, there was nothing in them inconsistent with the theory of a sex-linked recessive gene except, possibly, the fact that two sources of Light Sussex males were found to produce affected pullet chicks. These sources of cockerels must have been closely related, although this was not implied. It is unfortunate that carrier males were not mated to unrelated females having good reproduction so that the proposed inheritance of the character could be verified with appropriate numbers of chicks. Apparently stocks possessing the cerebellar hypoplasia were not preserved.

Comparable Conditions in Other Species

The paroxysmal syndrome is somewhat similar to an inherited condition of goats known for more than a half-century as the "nervous" or "fainting" goats. Unfortunately, in spite of the obvious hereditary nature of the condition, the genetic basis of the character has never been determined. Kolb[9], following the suggestion of Clark *et al.*[1], considered the disease to be a form of congenital myotonia. In many respects the present condition in the fowl is like that in the fainting goat. These similarities include, among others, a paroxysmal state precipitated by sudden fright and lasting 10 to 20 seconds, the unusual rigidity of muscles, absence of expression in the newly hatched (or born) animal, a refractory period between attacks, and the failure of all known affected animals to react at the same time. It differs by being lethal to the hemizygous female. Kolb[9] studied a nanny, a billy, and their two kids, one of each sex. This single family, all members of which were affected, would suggest that the condition is recessive and not lethal. He developed an ingenious method of inducing the syndrome in goats and established time relationships for the refractory period. Thus, he could determine the effect of various drugs upon the expression of myotonia. Some, such as quinine and some other cinchona derivatives, relieved the myotonia state (extended the refractory period) while others, such as prostigmin and pilocarpine, aggravated the symptoms by shortening the refractory period. Histologically the muscle tissue appeared to be normal.

Susceptibility to audiogenic seizures in mice is inherited as a dominant character[4]. More than one gene seems to be required for the character to be expressed. Resistance to death, that often follows the seizure, apparently depends upon other genes. The similarity of this trait to that of paroxysm is therefore limited to a seizure following audiogenic stimulation, which must be rather severe in the case of the mouse. The paroxysm lethal is also induced by other types of stimulation and death is not a direct result of the seizure.

The *xl* Lethal

Attempts were made to establish the locus for the *xl* gene, described by Goodwin *et al.*[5], but these were unsuccessful. Its presence in stock that carried dominant white (*I*) made it difficult to get suitable dihybrid males for linkage tests. Eventually two males, proven by test to be heterozygous for *B*, *S*, and *xl*, were obtained and used to measure linkage in their female offspring. The classification of some genes, especially *S* and *s*, could not be made with certainty, hence the data were not suitable to measure linkage with this particular sex-linked gene. In this series of 237 gametes tested, there was an expected ratio for $B:b$ of 119 to 118, but for the *xl* gene the ratio was 157 to 80, instead of equality. Not only did fewer of the pullets show symptoms of the lethal character, but most of them that did so survived to become normal adults. Obviously, during the course of matings used to obtain birds with appropriate sex-linked genes for linkage tests, there had been an accumulation of modifying genes which suppressed the action of *xl*.

If only those classes showing the *xl* phenotype are used to calculate linkage, the *xl* gene was 52.5 units away, or independent of *B*. If all the data are used, the crossing-over still remains at 52 percent. Further attempts to establish a more precise locus for *xl* were

abandoned because of decreasing penetrance of the lethal, even of the "sleepy" syndrome which often was the first or only expression of the gene.

Summary

A sex-linked recessive gene, *px*, causes paroxysm, poor growth, stilted gait, and eventual death in hemizygous females. The lethal gene appears to have no effect on hatchability or early growth. The first symptom, usually a paroxysm following a sudden auditory or visual stimulation, may occur as early as 12 days to two weeks, or as late as six weeks, and death will have occurred by 14 to 15 weeks, if not sooner. No gross or microscopic lesions have been observed.

The gene *px* is transmitted by heterozygous males independent of *B* (52.7 percent crossing-over) but closely linked to *n* (7.2 percent) and to *k* and *al* (11.6 and 10.5 percent crossing-over, respectively).

The sex-linked lethal gene, *xl*, is at least 52 cross-over units away from *B*.

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CHROMOSOME ABERRATIONS AND GROWTH HORMONE

BERKELEY, CALIF.—A team of scientists at the University of California have begun applying to human patients the fruits of many years of basic research on growth hormone.

In preliminary trials they have given growth hormone to an under-developed patient whose cells lack a female chromosome, and they have succeeded in stimulating physical growth.

The American Cancer Society, which for 15 years has supported studies of growth hormone here, recently reported on the progress of the research. The hormone is extracted from human pituitaries and purified by procedures devised by Drs. Choh Hao Li and Harold Papkoff of the Berkeley campus; its effects on cultured cells are tested by Drs. Henry D. Moon and Virginia L. Jentoft, and its effects on humans by Drs. Roberto F. Escamilla, John J. Hutchings and William C. Deamer, all of the University of California Medical Center at San Francisco.

During the last two years, widely scattered scientists have reported finding abnormal numbers of chromosomes in the cells of patients with several heretofore baffling congenital conditions — one daughter cell may wind up with 47 chromosomes, the other with 45.

The first chromosome case studied by the California group was a 14-year-old girl diagnosed as having Turner's syndrome. Female in appearance, she had only 45 chromosomes —an X-chromosome was missing. Girls with this condition grow very slowly, have underdeveloped breasts and other female physical characteristics, have web necks and may be mentally deficient. This patient, for sex glands, had only a fibrous remnant of one ovary.

While the scientists knew they could not cure the condition with hormones, they hoped they might increase the patient's growth rate —she was little more than four feet tall; and they gave her human growth hormone daily for more than three months. Under treatment, her growth rate doubled.

The results indicated that this single symp-

(*Continued on page* 88)

TEMPERATURE-SENSITIVE ALLELES OF THE Y_1 LOCUS IN MAIZE

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SINCE the effect of temperature upon mutant phenotypes is one of the easiest of the environmental variables to study, examples of temperature-induced modifications of mutants are fairly numerous. In rabbits, it has been shown that the Himalayan color pattern of white body and colored extremities is the result of a temperature-sensitive allele (c^h) of the albino series. (For review, see Robinson[47].) Studies have shown that the critical temperature is in the region of 25° C. Above this temperature very little or none of the enzyme necessary for melanin synthesis is formed, while below this temperature the enzyme is present in amounts sufficient for pigmentation to take place.

Extensive studies of temperature effects upon the expression of mutant phenotypes have been made in *Drosophila*. These included mutants at the vestigial,[1, 2, 20, 21, 22, 23, 24, 42, 46, 52, 53] bar eye[11, 25, 26, 30, 33, 49] and white eye[15, 29] loci.

Many temperature-sensitive mutants have been described in *Neurospora* that require their media supplemented with growth factors at one temperature (usually above 25° C.) and at other temperatures can grow without supplementing (usually at temperatures below 25° C.) [16, 17, 27, 28, 37, 39, 40, 41]. Similar biochemical, temperature-sensitive mutants have been described for other micro-organisms[31, 34, 35, 36].

In higher plants, Stanford[50] has described a chlorophyll-deficient mutant of alfalfa whose expression is influenced by temperature, and in barley, temperature-sensitive mutants have been described by Hallqvist[19], Collins[7] and Nybom[43].

In corn, the temperature-sensitive virescent mutants have been recognized since the early days of maize genetics. These mutants are yellowish or near white as seedlings, gradually turning green as the plants grow[6, 8, 12, 13, 32, 38, 44, 45]. Demerec[8] was able to demonstrate that high temperature accelerated the greening, and Phinney[44] has shown that the virescent mutant, pale-yellow-1, when grown in continuous light at 25° C., takes $7\,^2/_3$ days before the onset of greening, while normal plants require only one day. At 35° C., the onset of greening is observed in $1\,^5/_6$ days, which approaches the rate (half a day) of greening for normal plants grown at this temperature. The golden mutant of corn behaves in an opposite manner to the virescent. Usually golden seedlings are difficult to distinguish from normal, but when grown at high temperature and high light intensity this separation is sometimes possible[14]. Schwartz[48] has reported that the st^s allele of *st* is temperature sensitive. Plants homozygous for this allele do not show the mutant phenotype when grown at low temperatures (65° to 70° F.) but do when grown at high temperatures.

Description of Mutants

The two mutants used in this study are pastel-8549 (*pas-8549*) and white-mutable (w^{mut}). Seeds homozygous for the recessive *pas-8549* gene have white endosperm. Upon germination in the seedling bench, one of three phenotypes

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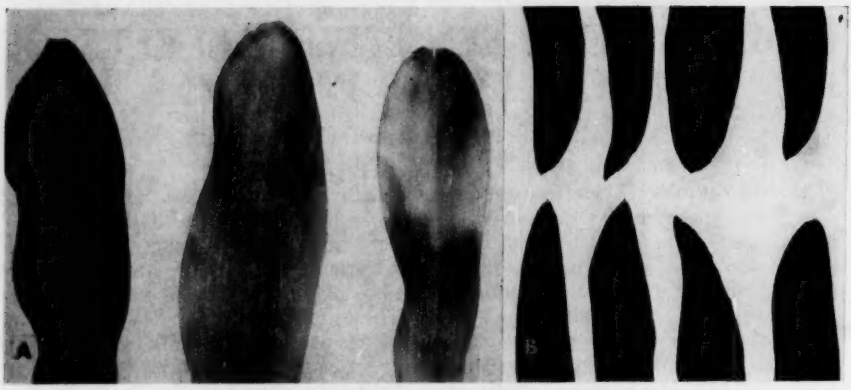

MUTANT Y_1 AND PASTEL-8549 LEAVES

Figure 2

A—Normal leaf, stable *w*-mutable leaf from plant grown at 37° C. and pastel-8549 leaf from plant grown in seedling bench during hot weather. The latter shows the zebra effect frequently observed in plants grown in the field or greenhouse where temperature is not held constant. *B*—Leaves from pastel-8549 seedlings and their normal sibs grown at 20°C. Normal leaves are shown above and mutant leaves below.

is observed, as the seedlings may: (1) be indistinguishable from normal seedlings, (2) appear pale green or (3) have a zebra phenotype (pale green with bands of paler green or white tissue transversing the leaf at more or less regular intervals (Figure 2*A*). Grown in the field, *pas-8549* mutants are considerably paler than normal plants and have a distinct zebra phenotype. The paler green cross-bands of the zebra pattern often approach albino tissue in their lack of pigmentation. Pastel-8549 plants also have white sheaths and leaves with pale green or white mid-ribs. Only an occasional *pas-8549* plant has been observed to live to maturity under field conditions, and these are extremely weak.

White-mutable is similar to *pas-8549* in phenotype. However, it is unstable and regularly back-mutates to normal in both endosperm and plant tissue. As a result, w^{mut} seeds have a white endosperm with spots of normal yellow tissue. The white-mutable plants grown in the field are pale green or zebra with streaks of normal green tissue (Figure 3). It has been possible to derive lines from w^{mut} stocks in which no back mutations are observed. In such stable lines the w^{mut} plants are indistinguishable from *pas-8549* mutants. Both mutable and stable homozygous w^{mut} plants seem to survive better under field conditions than *pas-8549* plants, and many have been grown to maturity. Some produce an ear and set seed.

Both of these mutants were first isolated at the California Institute of Technology by Dr. E. G. Anderson. Pastel-8549 originated in the progeny of a seed exposed to a gamma ray dose of 40,000 r units. White-mutable first appeared as a mutation in a F_1 cross of the mutant zebra-4301 with translocation $9\text{-}10_{4303}$. All 15 plants in the F_1 culture were self-pollinated. Selfed ears were homozygous yellow, except plant number nine, which segregated yellow and yellow-white mottled seeds. The yellow seeds gave normal green and yellow-green seedlings typical of *zb-4301*. The white mottled seeds gave white-mutable seedlings and some double recessives (w^{mut} and *zb-4301*), recognizable by their paler color. In later generations, plants without the *zb-4301* trait were selected.

Genetic Tests

Self-pollinated ears of the heterozygous plants, $Y_1/pas\text{-}8549$ and Y_1/w^{mut}, segregate in 3:1 ratios for yellow and white endosperm (Table I, crosses 1 and 2).

Crosses of the heterozygous plants, *Y_1/pas-8549*, to stocks homozygous for the recessive y_1 gene gave yellow and white seeds in 1:1 ratios (Table I, cross 3). The plants grown from the white seeds (*y_1/pas-8549*) of such segregating F_1 ears were green, and upon selfing produced white ears which segregate in a 3:1 ratio for green and pastel seedlings (Table I, cross 6). These results would indicate that *pas-8549* was allelic to y_1, and that the green plant condition of the y_1 gene is dominant to the pastel condition of *pas-8549*.

Crosses of the heterozygous plants w^{mut}/Y_1 to stocks homozygous for y_1 also segregated for yellow and white seeds in 1:1 ratios (Table I, cross 4). However, the white seeds show the areas of yellow tissue associated with the white-mutable gene. The plants grown from mutable seeds (y_1/w^{mut}) were normal green and upon selfing produced white ears with mutable and non-mutable seeds. One-fourth of the seeds from such an ear produced the pastel seedlings of w^{mut} (Table I, cross 7). These results indicate that w^{mut} is also an allele to y_1, and that w^{mut} is dominant to the y_1 gene in the endosperm (in stocks of w^{mut} where the mutable condition has been lost no dominance relationships in the endosperm can be recognized) and recessive in the seedling and mature plant.

White-mutable plants are very similar to a mutant described by Dollinger[9, 10]. Tests for allelism which he has made with his mutant have shown that it is also allelic to y_1 and pb_1. Dollinger presently is studying the mutable system operating at this locus.

WHITE-MUTABLE LEAF

Figure 3

A portion of a pale "zebra" stripe of a mature leaf from a white-mutable plant grown in the field. Notice the darker longitudinal stripes of normal green tissue where back mutation to the normal allele has taken place.

Crosses between homozygous w^{mut} plants and the heterozygous plants, *Y_1/pas-8549*, produced ears that segregate in a 1:1 ratio for yellow and white seeds with areas of yellow normal tissue (Table I, cross 5). The F_1 mutant plants show the w^{mut} phenotype and have its vigor.

TABLE I. Summary of segregations in crosses involving pastel-8549 and white-mutable

Cross	Phenotypes: Normal Obs.	Normal Exp.	Mutant* Obs.	Mutant* Exp.	χ^2	P
1. Self of *Y_1/pas-8549*	1013	1018.5	345	339.5	.1188	73%
2. Self of Y_1/w^{mut}	1634	1646.25	561	548.75	.3644	56%
3. $y_1\ y_1 \times$ *Y_1/pas-8549*	579	586	593	586	.6272	69%
4. $y_1\ y_1 \times w^{mut}/Y_1$ and $w^{mut}/Y_1 \times y_1\ y_1$	537	544	551	544	.1801	68%
5. *Y_1/pas-8549* $\times w^{mut}/w^{mut}$	493	507.5	522	507.5	.8286	38%
6. Self of *y_1/pas-8549*	563	564	189	188	.0071	94%
7. Self of y_1/w^{mut}	1168	1171.5	394	390.5	.0418	85%

*Mutant phenotype used in first five crosses was white endosperm. Pastel seedling was used in the last two crosses.

Temperature Experiments

After considerable trouble in consistently classifying mutant plants in the seedling bench, it became obvious that some environmental factor or factors were influencing the expression of the mutant phenotype. Since most of the difficulties in classification were encountered in the winter, and the best classifications were made in the spring or early summer, it was felt that temperature might be one of the important factors.

To test this hypothesis, seedlings were grown in a walk-in refrigerator with the inside dimensions of 6½′ × 5′ × 5′. The temperature regulation was by means of the refrigerator cooling system with a constant air blower for continuous circulation of the air. The refrigeration unit allowed a maximum variation of ±2° C. in temperature. The seedlings to be tested were grown in sand. Two fluorescent tubes served as the source of light with a resulting average light intensity of 115 foot candles as measured with a Norwood Director exposure meter, Model C. While this light intensity was low, it still was sufficient to cause strong greening of normal plants. Higher light intensities could not be used because of over-heating. The box was opened once or twice a day for watering and observation.

Before being planted, seeds from segregating ears were separated on the basis of endosperm color. White and yellow seeds were planted in adjacent rows, and after germination the seedlings from the two classes of seeds were compared for amount of greening. Both visual estimates of pigmentation in the intact plant and light absorption measurements of the extracted pigments were made of the seedlings grown at different temperatures. For the absorption tests, frozen seedlings were ground in a Waring blender in 100 ml. of 90 percent acetone for two minutes and the material filtered. Total chlorophyll was determined by the method of Arnon[4]. Chlorophyll of a 25 ml. aliquot of the extract was hydrolyzed with 10 ml. of KOH saturated ethanol for 30 minutes. After hydrolysis the material was poured into a separatory funnel, 20 ml. of Skelly A added, acetone-alcohol phase drained out, and Skelly A phase washed with water four times to remove acetone. The Skelly A phase containing carotene and xanthophyll was poured on a column (0.5 cm. × 5 cm.) of a 1:1 mixture of neutral alumina (Biochemical Corporation, Los Angeles, California) and anhydrous sodium sulfate. Carotene was removed from the column with two percent acetone in Skelly A as described by Booth[5]. Next, xanthophyll was removed with 80 percent methanol in water. The optical densities of the carotene and xanthophyll fractions were measured at 450 millimicrons, and the amounts of carotene and xanthophyll calculated, using 240 as the specific absorption coefficient[18]. At least two different xanthophylls were present in all of the samples;

TABLE II. Chlorophyll, carotene and xanthophyll analyses of normal, *pas-8549*, w^{mut} and F_1 seedlings at 37° C. and 20° C.

		37° C.			20° C.		
Ear no.	Allele	Chlorophyll (mg/gm f.w.)	Carotene (mg/gm f.w.)	Xanthophyll (mg/gm f.w.)	Chlorophyll (mg/gm f.w.)	Carotene (mg/gm f.w.)	Xanthophyll (mg/gm f.w.)
1937	*pas-8549* Nor.	.97	.0450	.0412	.86	.0298	.0511
1941	*pas-8549* Nor.	1.06	.0507	.0515	.88	.0320	.0391
1947	*pas-8549* Nor.	.95	.0478	.0353	1.24	.0407	.0650
1937	*pas-8549* Mut.	.33	.0034	.0052	.62	.0182	.0364
1941	*pas-8549* Mut.	.36	.0058	.0080	.66	.0268	.0345
1947	*pas-8549* Mut.	.28	.0049	.0146	.80	.0201	.0409
4967	w^{mut} Nor.	.61	.0321	.0294	.83	.0247	.0370
5068	w^{mut} Nor.	.51	.0267	.0332	.86	.0330	.0421
4967	w^{mut} Mut.	.11	.0022	.0031	.70	.0209	.0323
5068	w^{mut} Mut.	.13	.0023	.0038	.65	.0210	.0308
5140	F_1 Nor.	.62	.0332	.0296	1.05	.0261	.0521
5140	F_1 Mut.	.15	.0021	.0047	.82	.0156	.0266

one was present in a considerably greater concentration than the other. It is realized that the use of 240 as the specific absorption coefficient for the xanthophyll fraction is rather arbitrary.

Seedlings grown at 37° C. can readily be separated into mutants and normals. Visually, the mutant seedlings show a decided reduction in greening (Figure 2*A*). These differences are very marked when the pigment concentrations of the two classes are compared (Table II).

However, when plants were grown at 20° C., it was very difficult visually to separate consistently mutants from normal seedlings (Figure 2*B*), although one gets the impression that the mutants as a group are slightly lighter green than the normals. Determination of the total chlorophyll, carotene and xanthophyll contents of the seedlings shows that the concentration of these pigments in the mutants approaches that found in the normals (Table II).

Table II lists the chlorophyll, carotene and xanthophyll determinations for normal and pastel-8549, white-mutable and F_1 seedlings. The pastel-8549 seedlings and their normal siblings were grown for 151 hours at 37° C., while the white-mutable and F_1 seedlings were left eight additional hours before harvesting. The consistently lower values for the white-mutable and F_1 seedlings and their normal counterparts at this temperature are probably the result of these eight additional hours at 37° C. All seedlings were grown for 249 hours at 20° C. before harvesting.

Tables III and IV summarize values for chlorophyll, carotene and xanthophyll given in Table II. Table III summarizes the data for pastel-8549 seedlings and Table IV summarizes the data for white-mutable and F_1 seedlings. Pastel-8549 seedlings grown at 37° C. for 151 hours have 32.3 percent the chlorophyll content, 9.8 percent the carotene content and 21.8 percent the xanthophyll content of normals. However, when grown at 20° C. the chlorophyll content of the mutants is 69.7 percent that of normals, while the carotene content is 63.4 percent and the xanthophyll content is 72.1 percent that of the normals. The percentage values in Table IV are in rough agreement with those of Table III in spite of the marked differences in pigment levels at 37° C. of these two tables.

If one takes the behavior of the normal seedlings as standard, a marked temperature effect is observed in the concentration of chlorophyll, carotene, and xanthophyll in the pastel-8549 mutants (Table III). In normal seedlings

TABLE III. Summary of analyses of chlorophyll, carotene and xanthophyll for pastel-8549 seedlings grown at 37° C. and 20° C.

Pigment analyzed	Seedlings	37° C. Average pigment concentration mg/gm f.w.	37° C. Mutant / Normal	20° C. Average pigment concentration mg/gm f.w.	20° C. Mutant / Normal	Calc. mut. 20° C. (mut. 37°) / (Nor. 37° ÷ Nor. 20°) mg/gm f.w.	Obs. mut. 20° C. / Calc. mut. 20° C.
Chlorophyll	Normal	.99	32.3%	.99	69.7%	$\frac{.32}{1.0} = .32$	2.2
	Mutant	.32		.69			
Carotene	Normal	.0478	9.8%	.0342	63.4%	$\frac{.0047}{1.4} = .0034$	6.4
	Mutant	.0047		.0217			
Xanthophyll	Normal	.0427	21.8%	.0517	72.1%	$\frac{.0093}{.83} = .0112$	3.3
	Mutant	.0093		.0373			

TABLE IV. Summary of analyses of chlorophyll, carotene and xanthophyll for w^{mut} and F_1 seedlings grown at 37° C. and 20° C.

Pigment analyzed	Seedlings	37° C. Average pigment concentration mg/gm f.w.	37° C. Mutant / Normal	20° C. Average pigment concentration mg/gm f.w.	20° C. Mutant / Normal	Calc. mut. 20° C. (mut 37°) / (Nor. 37° ÷ Nor. 20°) mg/gm f.w.	Obs. mut. 20° C. / Calc. mut. 20° C.
Chlorophyll	Normal	.58	22.4%	.91	79.1%	$\frac{.13}{.64} = .20$	3.6
	Mutant	.13		.72			
Carotene	Normal	.0307	7.2%	.0279	68.8%	$\frac{.0022}{1.1} = .0020$	9.6
	Mutant	.0022		.0192			
Xanthophyll	Normal	.0307	12.7%	.0437	68.4%	$\frac{.0039}{.70} = .0056$	5.3
	Mutant	.0039		.0299			

the chlorophyll level at 37° C. is 1.0 times the level at 20° C. If temperature had the same effect on the mutant, a chlorophyll concentration of .32 mg/gm f.w. would be expected (Table III, column 7) at 20° C. The value of .69 mg/gm f.w. observed for the mutant at this temperature is 2.2 times higher than expected. The carotene level of normal seedlings at 37° C. is 1.4 times the level at 20° C. Based on this figure, the carotene level expected in the mutant at 20° C. would be .0034 mg/gm f.w. (Table III, column 7). The value of .0217 mg/gm f.w. observed for the mutant at 20° C. is 6.4 times higher than expected. There is a similar effect of temperature on the production of xanthophyll in the mutant. At 20° C. a xanthophyll level of .0112 mg/gm f.w. is expected (Table III, column 7) instead of the observed .0373 mg/gm f.w., which is 3.3 times higher than expected. The observed values for chlorophyll, carotene and xanthophyll for white-mutable and F_1 seedlings grown at 20° C. are also higher than expected (Table IV). The observed values differ from the expected by even larger amounts than in the case of the pastel-8549 mutants. These greater deviations reflect the relatively greater effect that the eight additional hours at 37° C. had upon pigment production in mutants than in the normal seedlings.

Discussion

The results of the temperature experiments would indicate that *pas-8549* and w^{mut} are temperature-sensitive mutants. Their response to temperature is just the opposite of the temperature-sensitive virescent mutants. Phinney[44] found that virescent *v-pg*$_1$ greened at high temperatures and remained pale at low temperatures. However, the temperature response of these mutants is similar to that reported for golden seedlings by Emerson *et al.*[14] and by Schwartz[48] for the *st*° mutant.

The response to temperature of these mutants is also similar to that of several microorganisms. While the complete action spectrum of the corn mutants over the range of viable temperatures has not been determined, it is obvious that the temperature effect is in the same direction (high temperature for expression of mutant phenotype). However, most temperature-sensitive mutants of microorganisms show very little or no growth at high temperatures, indicating a highly efficient block of biosynthesis under these conditions, while these mutants of corn are only partially blocked at high temperatures. In this respect, these mutants are most similar to the temperature-sensitive blood allele (w^{b1}) of the white eye series in *Drosophila* where the block is not complete at high temperatures[15, 29].

A plant homozygous for the *pas-8549* or the w^{mut} allele is deficient in both chlorophyll and carotenoid pigments. Just how the mutant gene affects the levels of two such diverse pigments is not clear from these preliminary studies. If one assumes only one function for the normal allele at this locus, then there are

at least three explanations that fit the present data for the simultaneous involvement of the chlorophyll and carotenoid pigments in the mutants:

1) The mutant gene might result in changes in the chloroplasts which would affect their pigment-carrying capacity. Since both carotenoids and chlorophyll are found in the chloroplasts, such a defect might alter the level of both of these pigments.

2) It is possible that the gene controls a step common to the biosyntheses of carotenoid and chlorophyll. Some step in the synthesis of the polyisoprene carbon chain which is common to both the carotenoid molecule and the phytol part of the chlorophyll molecule might be involved.

3) Recent work on the role of carotenoids in protecting chlorophyll might provide an alternative explanation for the simultaneous involvement of these pigments in these mutants. Stanier[51], working with photo-synthetic bacteria, has suggested that carotenoids protect against the photo-auto-oxidation of chlorophyll, and Anderson and Robertson[3] have evidence suggesting that carotenoids might have a similar function in corn. If these conclusions are substantiated by further work, the results reported here might be due primarily to the temperature sensitivity of carotenoid synthesis in the mutant which only secondarily affects the chlorophyll level. The fact that in the mutant the carotenoid levels are more drastically altered than are chlorophyll levels would suggest that the primary effect of the gene might be in carotenoid synthesis.

It is possible that a combination of these mechanisms might be needed to explain the observed pigment levels or that some entirely different explanation might be necessary. Further work is planned to determine more accurately the behavior of pastel-8549 and white-mutable seedlings under environmental conditions that should give clues as to the mechanism responsible for altering both the chlorophyll and carotenoid contents in these mutants.

Summary

The response to temperature of two y_1 alleles, *pas-8549* and w^{mut}, is described. In addition to the y_1 phenotype, plants homozygous for these alleles are pale green as seedlings and as mature plants when grown at high temperatures. Grown at low temperatures, mutant plant color approaches normal. Absorption analyses of extracted pigments from normal and mutant plants grown at 37° C. and 20° C. reveal that compared to normals, the levels of chlorophyll, carotenoid and xanthophyll in the mutants at the lower temperature are substantially higher than expected. Observed concentrations of these pigments in the mutants at 20° C. were higher than expected by factors of 2.2 — 3.6 for chlorophyll, 6.4 — 9.6 for carotene and 3.3 — 5.3 for xanthophyll. Mechanisms which would explain the simultaneous effect of these alleles on chlorophyll and carotenoid contents are discussed briefly.

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VIABILITY AND REPRODUCTIVITY OF SUPERFEMALES IN DROSOPHILA

GERDA ROLFES AND W. F. HOLLANDER*

THE discovery of non-disjunction of sex chromosomes by Bridges about 1913 as a rare error in meiosis indicated that an individual could be produced having three X-chromosomes. He later[3] obtained such specimens from "high non-disjunction" families and also from triploids, and named them "super-females." By 1925[10] he no longer hyphenated the term. Along with intersexes having two X-chromosomes and triploid autosomes, they were part of the evidence on which he rested his balance theory of sex determination. That is, it is not the absolute number of X-chromosomes which controls the sex, but the proportion of X to autosomal material.

Actually the triplo-X "superfemales" proved to be a very inferior or sublethal type. From attached-X mothers about one-fourth of the zygotes are expected to be triplo-X, and one-fourth with no X (lethal). It was shown by Morgan[8] in 1925 that such matings usually produce less than three percent superfemales; however, several cultures were exceptional in yielding much closer to the expected 33 percent.

Dobzhansky[6] reported in 1928 that temperature or some associated variable was a critical factor for viability to the adult stage. The optimum temperature was 20°C., at which he obtained five percent superfemales. Higher and lower temperatures (30.5°, 24.5°, and 16°) gave much lower frequencies. Dobzhansky observed no correlation between degrees of larval crowding and percentage of emerged superfemale adults.

By using certain color mutants as markers it is possible to identify superfemales in the larval stage. Brehme[2] used this method to determine time of death. About one-third died as larvae, and nearly two-thirds as pupae. The emergence of superfemale adults in her cultures was three percent ±0.9 percent of the total larvae from attached-X mothers. It has also been found that superfemale larvae pupate a day or more later than normal females, and are slow-developing in the pupa stage. Emergence is therefore usually several days late[4].

There is agreement in the literature that superfemales are always sterile. Morgan *et al.*[10] stated that the ovaries are abnormal. Beadle and Ephrussi[1] cited Dobzhansky's studies and agreed that the ovaries ordinarily never give rise to fully developed eggs. However, Beadle and Ephrussi succeeded in getting eggs by transplanting ovaries from superfemale larvae into normal female larvae. In genetically female-sterile (*fes*) hosts several thousand eggs were produced, of which only 30 became adults. Only one of these 30 was clearly a cross-over product; two others were patroclinous males, and another apparently haplo-IV, indicating a high frequency of non-disjunction. The authors concluded: "Since there are no marked irregularities in somatic development of a superfemale individual that would indicate disturbances in mitotic cell division, ... the particular chromosome unbalance characteristic of such individuals has a specific effect on the meiotic mechanism."

Medvedev[7] also reported success in obtaining progeny from transplanted superfemale ovaries.

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Heterosis Tests

The following study was originally outlined to determine whether the viability of superfemales could be increased by heterosis. The stocks employed are listed in Table I. All except "II" were closed stocks (maintained for five or more years without crossing) but not deliberately sib-mated. A simple cornmeal, molasses, brewers yeast, agar medium was used, without mold inhibitor other than the actively growing yeast inoculum. Temperature in the laboratory ranged from 21° to 25° C. Unless otherwise noted, six females were used per culture bottle.

The first experiment consisted of removing all advanced pupae (near emergence) from culture bottles of attached-X matings, until no more appeared. These pupae were removed by first wetting them with a brush, to loosen them from the glass, and after a minute or two picking them off with needle and brush. Next they were examined (wet) under a binocular dissecting microscope (×15) and classified as male, female, or superfemale by means of the sex combs and color markers visible through the pupal case. The three groups were placed separately in fresh bottles and emergence recorded two days later (Table II).

Since the temperature during this test was generally "good," about 22° C., the very poor emergence frequencies of superfemales may signify greater sensitivity of these to the handling procedures. The low emergence figure for males of stock Q may have a similar explanation. But crossing apparently did improve the percentage of superfemales arriving at the advanced pupal stage.

A second experiment was set up to extend the observations to additional crosses, but instead of the pupae the emerged flies were removed daily (Table III). Each cross was started in two bottles, with six females per bottle (exception: W × L, one bottle).

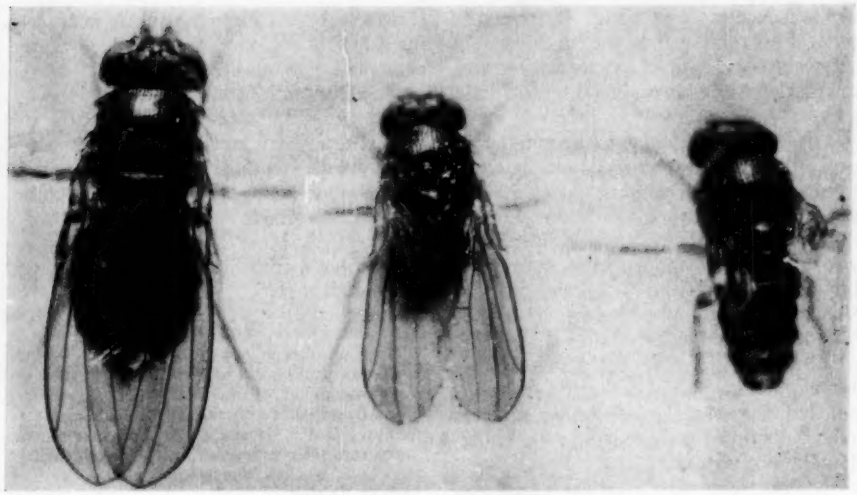
TABLE I. *Drosophila* stocks employed

Designation	Description or markers	Gene symbols
I.	synthetic wild stock	+
II.	captured wild stock (Ames, 1959)	+
A	white eye, miniature wing	*w m*
B	bar eye	*B*
C	white, forked bristles, curled wing	*w f; cu*
D	crossveinless, bar, dumpy wing	*cv B; dp*
E	white eye, ebony body	*w; e*
F	white eye, forked	*w f*
K	scute bristles, crossveinless, vermilion eye, forked	*sc cv v f*
L	crossveinless, vermilion, forked, lobelike eye	*cv v f*
M	(females attached-X, yellow body), males miniature-wing dominant	m^D
Q	females attached-X, yellow; males crossveinless, bar	*yy, cv B*
R	raspberry, vermilion eye, forked	ras^2 *v f*
S	white eye, singed bristles	*w* sn^3
T	white, ebony body, taxi wing	*w; e tx*
V	vestigial, white, ebony, eyeless	*w; vg; e; ey*
W	females attached-X, yellow; males white eye	*yy, w*

TABLE II. Ability of flies to emerge from counted pupae

Parents ♀	Parents ♂	Male pupae No.	Male pupae % emerged	Female pupae No.	Female pupae % emerged	Superfemale pupae No.	Superfemale pupae No. emerged	Total pupae	Superfemales % of total
W	W	437	90.2	234	91.0	21	2	692*	3.0
Q	Q	316	59.4	304	99.0	5	–	625*	0.8
W	F	201	96.0	153	98.0	40	–	394	10.2
W	E	227	97.4	182	98.0	49	–	458	10.7
Q	E	123	97.6	133	99.2	18	–	274	6.6
Q	B	372	93.9	374	98.7	57	3	803*	7.1
W	S	231	98.7	159	100.0	29	–	419	6.9
W	I	309	96.8	326	98.8	39	–	668*	5.8

*Two bottles used.



NORMAL AND SUPERFEMALES

Figure 4

A normal female (left) and two divergent examples of viable superfemales. Typical features are small size, wings scalloped on inner margins or more severely deformed, and crumpling of hind legs.

Although the large numbers of viable male and female hybrids produced in these crosses indicate that heterosis is involved, certainly the proportion of superfemales emerging does not show a general improvement.

Why do so few of the superfemales reaching advanced pupal stages fail to emerge? Since a good deal of variation in structure of these flies had become apparent (contrary to the statement of Beadle and Ephrussi, quoted above), careful examinations were made. For this purpose four crosses were chosen, more or less randomly, and 30 superfemale pupae taken from each. A summary of the abnormalities is given in Table IV.

Abnormal head structure, occurring chiefly in specimens from Q × B and W × D, seems probably favored by the Bar factor. Similarly, eyes with

TABLE III. Emergence frequencies of superfemales in various crosses

Parents ♀	Parents ♂	Males	Females	Superfemales	Total flies	% Superfemales
Q	W	714	623	4	1341	0.3
Q	E	672	612	2	1286	0.2
Q	T	516	504	2	1022	0.2
Q	V	229	387	–	616	0.0
Q	B	389	372	3	764*	0.4
Q	II	896	878	4	1778	0.2
W	M	810	767	3	1580	0.2
W	Q	366	364	–	730	0.0
W	D	743	689	–	1432	0.0
W	A	679	704	5	1388	0.4
W	R	691	672	7	1370	0.5
W	C	712	697	3	1412	0.2
W	II	915	824	10	1749	0.6
W	L	424	418	48	890**	5.4

*Three dams died early.

**Only one bottle.

white centers seem to be encouraged by the presence of a *w* gene. However, in general no relationship was discoverable between the kind of abnormality and the marker genes introduced by the male parent.

There is a tendency to symmetry in the individual, but correlation between different sorts of abnormalities is unpredictable. Death may depend on the degree of local necrosis. Specimens with a well-developed abdomen may have shrunken (dead?) thorax, and vice versa. In this connection it should be noted that Medvedev[7] found that transplanted imaginal discs from superfemale larvae to normals developed normally, so that a general or systemic fault in metabolism probably exists in the superfemale organism.

Ability to emerge from the pupal case seems to be dependent on adequate development of head, thorax, and abdomen. The high frequency of leg deformities seems a great handicap. Practically all superfemales which emerged showed more or less clumsiness in walking, and often one or more legs were crumpled.

The illustration of a superfemale given by Morgan *et al.*[10] (page 158) in our experience is not typical. The wings are shown extending far beyond the end of the abdomen, whereas they actually are usually much shorter, and flight is impossible. Most specimens are puny, and all have conspicuously irregular eye facets ("rough").

Fertility

Because of the relative success of W × L in producing viable superfemales, the cross was propagated to F_2. Again many superfemales emerged. In F_3, F_4, and F_5 the viability was still better, despite average room temperature of 24°C. Moreover, these superfemales tended to be less abnormal than usual. Especially in F_3, F_4, and F_5 some were nearly normal females in appearance and action. These individuals were placed in fresh culture bottles with males of the father's type. It was found that they often mated, but as a result of the weakness of their legs they tended

TABLE IV. Abnormalities of superfemales in late pupal stage (30 individuals examined in each cross)

Features	Q × B	W × D	W × A	W × H
Legs distorted	25	28	18	20
Legs or parts of legs missing	6	6	1	1
Wings small or abnormal shape	21	26	17	19
Wings missing	9	7	–	4
Bristles defective (missing, forked, malposition)	17	13	12	16
Bristles doubled	3	11	3	4
Thorax shrunken or deformed	19	6	7	15
Scutellum very small	1	1	2	6
Ocelli abnormal position or missing	4	5	2	2
Eyes partly white (centrally)	1	1	11	–
One eye missing	–	4	–	–
Head shape or structure abnormal	20	12	6	–
Abdomen shrunken or irregular	1	11	3	3

TABLE V. Progeny of superfemale

Markers visible (phenotype)	Females				Males			
	total	killed	died	bred	total	killed	died	bred
wild type	36	4	2	30	4		1	3
cv v f	16	1	4	11	11	7	2	2
v f	5	2		3				
f	3		1	2				
y	17		6	11	8	1		7
y cv v f					4			4
y v f					10	4	2	4
y f					3	1	1	1
y v					2		1	1
Total	77	7	13	57	42	13	7	22

when copulating to get stuck in the food.

The "best" superfemales, about 30 in all, were transferred each third day to fresh bottles. After the second transfer some eggs were laid, perfectly normal in appearance, but ordinarily none hatched. However, one healthy specimen from the F_5 broke the rule, giving 119 progeny. She had only slightly scalloped wings and rough eyes. The progeny are tabulated in Table V.

The phenotypes of these progeny clearly show that the mother had three sex chromosomes and that there was no restriction of crossing-over such as Beadle and Ephrussi found.

Some of the wild-type daughters should have been superfemales again, but they showed no wing defects and were vigorous. Progeny tests of these as well as many of the other types were made individually with males of stock K (see Table V, column headed "bred"). Ten of the wild-type females proved to be attached-X, giving patroclinous sons. The remaining 20 bred poorly, and produced various types of sons as well as some yellow daughters and intersexes. Therefore, it was concluded that they were triploid. Further tests with autosomal markers verified this conclusion.

In view of the phenotypic abnormalities of the fertile superfemale, as well as the rather large number of progeny, she could hardly have been a triploid herself. Bridges[10] (page 162) noted that sometimes normal females and also intersexes have "cysts" of polyploid cells in the ovaries. Possibly something similar occurred in the case of the superfemale. It is difficult to explain the apparent absence of intersexes among her progeny. Possibly they were present and marked as males or females because of inexperience in classifying; they would probably then be in the column headed "died".

Repetitions of the original W × L cross have confirmed the finding that high percentages of viable superfemales result. Many of these were isolated with males, and some laid eggs again, but none hatched.

Among the descendants of the original fertile specimen, over 400 attached-X females have been individually tested. The great majority yielded a very low percentage of viable superfemales, if any. However, at least two separate cases were much more successful, and successive cultures continued to produce well. Of approximately 150 superfemales maintained for possible breeding, one again was fertile.

This second fertile example had been sired by a bar-eye male (stock B), so that roughness of her eyes was not clear. The wings were frayed at the tips, not scalloped. She was fairly vigorous and not small. Mated with a special sex chromosome marker male (Muller's "Base"), she produced 121 progeny of many phenotypes. Some were triploids, and some diploid and/or attached-X females, as determined by progeny tests. There were also intersexes and fertile males, showing evidence that abundant crossing-over had occurred. Five of the males were patroclinous and fertile, indicating that these obtained a Y-chromosome from the mother.

In this second fertile superfemale instance we are faced with a further problem of interpretation. The somewhat abnormal phenotype and relatively high reproductivity again do not well support the idea that she herself was a triploid, but she produced triploid daughters and intersexes. In the case of the previous example it was not possible to determine whether a Y-chromosome was included. The fact that the second apparently did have a Y-chromosome tempts us to suggest that it may be an important factor in producing viable superfemales. Further tests of this and other hypotheses could not be included in the present study.

Summary

Frequency of superfemale *Drosophila* produced by attached-X females and arriving at late pupal stage and emerging as adults was studied in two closed stocks and in crosses with males of unrelated stocks. Abnormalities were extremely varied and irregular. Crossing produced an apparent improvement in viability of superfemales until the late pupal stage, but little or none in percentage of emergence. However, superfemales were much more regularly successful from one cross, and the viability increased in F_2 through F_5. Contrary to previous workers' observations, temperature is a less important variable than genotype or other unknown factor. Over 200 superfemales with above average viability have been maintained with males. A fair proportion laid some eggs, and two produced progeny, 119 and 121, respectively. In both cases a considerable number of triploid daughters resulted, and crossing-over was not inhibited. Patroclinous sons indicated that at least one of these fertile examples had also a Y-chromosome. The possibility that the fertile superfemales were mosaic for triploid tissue is considered. It is suggested that the extra Y-chromosome may be a factor in increased viability.

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PRINCIPLES OF HUMAN GENETICS

THE first edition (1949) of this book supplied a text which presented all basic genetic principles through a consideration of the genetics of man. This new edition* serves the original purpose even more fully by inclusion of an imposing amount of new material reflecting the considerable advances in genetics as a whole, and of our knowledge of inheritance in man in particular. As the preface suggests, there have been "breath-taking changes and advances" in this field in the last decade. Additions have been skillfully organized and incorporated with the original text. While a number of whole, or part, chapters remain essentially unchanged, rewriting, some reorganization, and expansion make this a new book in many respects.

The book is divided into 33 chapters, rather than the original 27. "Linkage and Crossing Over," formerly a chapter of 22 pages, has been expanded into two chapters covering a total of 43 pages. The old chapter on "Heredity and Environment" (52 pages) is broadened —in 72 pages—into three chapters subheaded, respectively: I. Types of Twins, II. Physical Traits, and III. Mental Traits. Also, the two original chapters on "Mutations" are increased to three (and an additional 30 pages) partly by separating into a third chapter, "The Genetic Hazards of Radiation." "Selection and Eugenics" has been largely rewritten and expanded into the resulting chapters on "Selection and Genetic Polymorphism" and "Selection in Civilization." Another, added, chapter deals with "Aspects of Medical Genetics."

Along with, or in addition to, the new chapters there are 136 pages, 25 tables, 67 figures and 38 problems *more* than were found in the first edition. Moreover, a different—and very readable—type adds some 20 percent to the numbers of words per page. As might be expected from this, considerable new material has been added to many of the original chapters. For example, Chapter 2 now includes an excellent discussion of the recent work on human chromosome numbers and types; Chapter 3 (Genic Action) has an added section on "Human Embryology"; Chapter 5 (Probability) adds a brief but lucid section on "Correlation" —and so on through the book.

Restatements and careful arrangement have coordinated all new, with old, material in such a way as to increase the forcefulness of the

(*Continued on page* 82)

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DIFFERENTIAL SENSITIVITY TO GAMMA IRRADIATION OF LILIUM VARIETIES

GURU D. PERSHAD AND C. C. BOWEN*

VARIATIONS in response to radiation among varieties of the same plant species and among species of the same genus are of considerable interest as a starting place for investigations of the fundamental nature of radiation reaction. A number of investigations have demonstrated specific and even varietal differences in radiation response. Abrams[1] and Gonzalez and Frey[8] germinated irradiated seeds of several varieties of oats and found significant differences in sensitivity. Sparrow and Christensen[13] observed plants grown in the vicinity of a Cobalt-60 source and reported considerable differences in the radiation tolerance of different species.

The most studied differences in radiation reaction between species in the same genus have been those which can be correlated with variations in chromosome number. Sparrow[12] showed that in polyploid series within *Chrysanthemum, Sedum, Graptopetalum,* and *Luzula* the higher the ploidy levels the greater tolerance to radiation. He also pointed out that chromosome size seems to play an important role in determining radiation sensitivity. All the plants with large chromosomes that he studied had relatively high radiation sensitivity as compared to plants with small chromosomes. Sensitivity in these experiments was determined by the level of chronic gamma irradiation at which plant growth was visibly deranged.

On the other hand, in certain genera radiation sensitivity cannot be correlated with chromosome size or chromosome number. Johnson[9] showed differences in X-ray sensitivity in three varieties of *Atriplex hortensis* which she correlated with varietal variations in catalase activity. Smith[11] demonstrated that the presence of a recessive Mendelian gene greatly decreased growth rate in seedlings of *Triticum monococcum* after X-ray irradiation of dormant seeds homozygous for this factor.

The majority of species of *Lilium* have the same chromosome number ($2n=24$) and have similar chromosome sizes. As first shown by Erickson[7], a close relation exists between the stage of gametogenesis and bud length in *Lilium*. In this genus measurement of bud length permits determination of stages within narrow limits without the necessity of examining the sporogenous tissue microscopically. The anthers of *Lilium longiflorum* var. "Croft" have been utilized by several workers[4, 5, 10] in their radiation experiments. Lilies would thus appear to be an excellent material for the investigation of varietal and specific differences in radiation reaction, divorced from possible effects of varietal differences in karyotype.

Data from the experiments reported here throw light on a number of cellular responses to radiation other than varietal and specific differences. Discussion of these aspects will be published elsewhere.

Material and Methods

These experiments were done with eight horticultural varieties of *Lilium*—

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"Enchantment," "Midcentury," "Creelman," "Regale," "Ace," "Croft," "Erabu," and "Estate." All varieties have a chromosome number of $2n = 24$ and their botanical names and origin are as follows[6,14]: "Enchantment" and "Midcentury" are selections made from *L.* × *hollandicum* Berg (*L. bulbiferum* Linn × *L.* × *maculatum* Thun. (*L. dauricum* Ker-Gawler × *L. concolor* Salis.)) × *L. tigrinum* Ker-Gawler; "Creelman" is *L.* × *imperiale* Wilson (*L. regale* × *L. sargentiae* Wilson); "Regale" is *L. regale* Wilson; "Ace," "Croft," "Erabu" and "Estate" are *L. longiflorum* Thunb.

Pilot studies were made to determine the size of the bud at given stages of meiosis. The data obtained indicated that the stage of a bud of a given variety could be approximately determined by bud length measurement.

In Experiment 1 excised buds of varieties "Enchantment," "Midcentury," "Creelman," and "Regale" were irradiated at 94r at diakinesis by exposure to Cobalt-60 gamma source for six minutes. The buds were then kept on moist filter paper in petri dishes. After eight hours in a moist chamber, buds were dissected, sporocytes were squeezed out of the anthers into fixative, stained by the Feulgen technique and mounted according to techniques described by Bowen[2,3].

In Experiment 2 anthers of the four varieties of *L. longiflorum* were dissected out of buds at both pachytene and diakinesis, as determined by bud length. Anthers were placed in small vials and were exposed to 20r, 40r, and 60r of Cobalt-60 irradiation at the same dose rate as in the first experiment. Anthers were then floated on a sterilized culture medium consisting of one part coconut milk and eight parts of two percent sucrose solution in petri dishes at 60° F. in the dark. Anthers were cultured for eight to nine hours after diakinesis irradiation and for 70 to 72 hours after pachytene irradiation. The sporocytes were then fixed, stained and mounted using the same methods as in the first experiment.

Material found to be in meiotic anaphase I after eight to nine hours was assumed to have been in diakinesis and after 70 to 72 hours was assumed to have been in pachytene at the time of irradiation. All other slides were discarded. One slide per bud per dose was coded and scored under oil immersion. The number of normal cells as well as the numbers of bridges and fragments in cells at anaphase I were recorded. A minimum of three buds of each variety for each stage and dose were scored

Experimental Results

The mean number of bridges and fragments per cell and the bridge frequency distribution per cell observed in Experiment 1 are tabulated in Table I. The means given in the body of the table are weighted averages, and the intervals are 95 percent confidence limits based on the t-distribution. It will be noted that "Enchantment" and "Midcentury" (for convenience designated as Group 1 varieties) appear to be several times more sensitive than "Creelman" and "Regale" (Group 2). There also appears a marked difference in reaction to irradiation for the distribution of bridges per cell between these two groups. Group 1 varieties show up to five bridges per cell, as opposed to a maximum of two bridges per cell in

TABLE I. Mean values of bridges and fragments per cell in four varieties of *Lilium* irradiated with 94r at diakinesis in Experiment 1

Varieties	Fragments per cell	Bridges per cell						
		0	1	2	3	4	5	Mean
Enchantment	.10±.04	210	170	114	49	24	5	1.16±0.50
Midcentury	.08±.05	280	190	135	65	25	3	1.10±0.41
Creelman	.05±.01	876	163	15	0	0	0	0.18±0.03
Regale	.04±.03	317	33	5	0	0	0	0.12±0.03

Group 2 at the same dose. The mean values of bridges per cell points up a third difference. Group 1 exhibits a very wide bud variation in this respect, while the data for the several buds examined in Group 2 appear to be much more consistent. A homogeneity chi-square test for the bridge frequency distribution per cell in this same experiment is given in Table II. This test suggests similarity of frequency distributions for the varieties within each group. However, there is strong evidence against the hypothesis that the frequency distributions are the same in the two groups. Fragment frequency parallels bridge frequency and appears to substantiate the observation that varieties in Group 1 are more sensitive than the varieties in Group 2.

The mean number of bridges and fragments per cell observed in Experiment 2 are tabulated in Table III. It will be noted that all four varieties of *L. longiflorum* appear to be equally sensitive at both pachytene and diakinesis for bridges and fragments at all doses. These values are plotted against dose in Figure 5. The four varietal curves for a given stage for bridges and fragments were tested for coincidence in Table IV and were found not to deviate significantly except for fragment data at diakinesis. Apparently the F-test showed significance in this latter case because the four varieties individually showed

TABLE II. Homogeneity chi-square test for four varieties of *Lilium* irradiated with 94r in Experiment 1

Varieties		Bridges per cell at diakinesis					
		0	1	2	3	4	5
Enchantment	Ob.	210	170	114	49	24	5
	Ex.*	220.7	162.1	112.2	51.3	22.1	3.6
Midcentury	Ob.	280	190	135	65	25	3
	Ex.	269.3	197.9	136.9	62.6	26.9	4.4
Group 1	Ob.	490	360	249	114	49	8
	Ex.	797.8	263.6	127.5	54.0	23.2	3.8
Creelman	Ob.	876	163	15			
	Ex.	892.4	146.6	15.0			
Regale	Ob.	317	33	5			
	Ex.	300.6	49.4	5.0			
Group 2	Ob.	1193	196	20	0	0	0
	Ex.	885.2	292.4	141.5	60.0	25.8	4.2

^{2}within groups:
Group 1 — $\chi^2(5) = 3.18$
Group 2 — $\chi^2(2) = 8.46$†
$\chi^2(5)$ for groups $= 702.67$‡

*Ex—expected value on the basis of homogeneity.
†Significant at five percent level.
‡Significant at one percent level.

TABLE III. Mean values of bridges and fragments per cell in four varieties of *L. longiflorum* after pachytene and diakinesis irradiation in Experiment 2

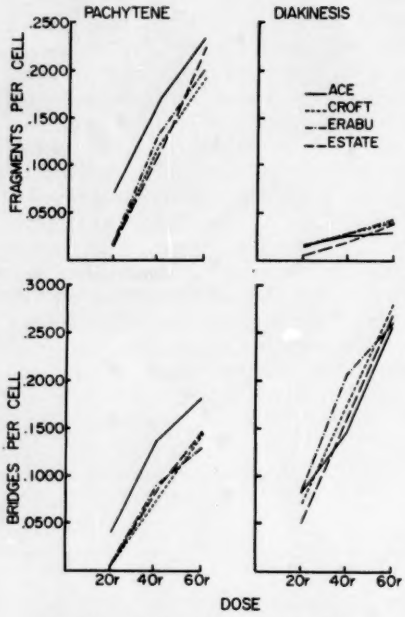
Varieties	Bridges			Fragments		
	20r	40r	60r	20r	40r	60r
			Pachytene			
Ace	.04±.01	.13±.02	.18±.02	.07±.09	.17±.11	.23±.11
Croft	.01±.01	.07±.02	.14±.02	.02±.08	.12±.01	.19±.09
Erabu	.00±.00	.09±.01	.13±.01	.02±.02	.13±.14	.20±.11
Estate	.01±.01	.08±.03	.14±.02	.01±.05	.11±.08	.22±.10
			Diakinesis			
Ace	.08±.02	.15±.03	.25±.05	.02±.04	.03±.04	.03±.04
Croft	.07±.02	.17±.04	.28±.04	.02±.03	.03±.04	.04±.03
Erabu	.08±.02	.21±.03	.26±.05	.01±.02	.03±.04	.04±.04
Estate	.05±.01	.16±.10	.27±.08	.01±.04	.02±.02	.04±.01

unusually good accord with the model assumption, i.e., the data fell almost exactly on four straight lines, and thus gave rise to a very small estimate of error. The F-tests carried out were standard linear hypothesis tests of the hypothesis that a single population regression line underlies all 12 observations, given the model assumption that possibly different population regression lines underlie each of the four varieties.

Bridge frequency distribution per cell found in Experiment 2 was compared for all varieties at all doses of irradiation at pachytene and diakinesis in Table V. The homogeneity chi-square test showed no differences in distribution among varieties except for the data obtained at 20r irradiation at pachytene.

Discussion

On the basis of these results, it is reasonable to consider "Enchantment" and "Midcentury" as varieties which behave similarly in several respects, and these then have been designated as Group 1 varieties. Similarly "Creelman" and "Regale" have apparently much in common and are designated Group 2 varieties. Group 1 varieties are considerably more sensitive than those in Group 2. Group 2 varieties exhibited much greater variation in mean bridge frequency between buds than Group 1. Finally, Group 1 varieties show up to five bridges per cell as opposed to a maximum of two bridges per cell in Group 2 at the same dose. This cannot be entirely accounted for by the greater sensitivity of Group 1 varieties, since the bridge frequency distribution in Group 1 was significantly different from the distribution in Group 2 (Table II).



DOSE ABERRATION CURVES

Figure 5

Bridges and fragments per cell at three doses of irradiation at pachytene and diakinesis in four varieties of *Lilium longiflorum*.

It was shown that four horticultural varieties of *L. longiflorum* showed no significant differences with respect to sensitivity at three dose levels at two stages (Table IV). The

TABLE IV. Test of coincidence of dose-aberration curves in four varieties of *L. longiflorum* in Experiment 2

Varieties	Bridges			Fragments		
	a*	b†	$F_{(a,4)}$	a	b	$F_{(a,4)}$
			Pachytene			
Ace	.1186	.0700		.1578	.0812	
Croft	.0749	.0678		.1097	.0866	
Erabu	.0737	.0631	3.87	.1177	.0910	4.82
Estate	.0775	.0696		.1161	.1038	
Mean	.0862	.0676		.1254	.0906	
			Diakinesis			
Ace	.1609	.0868		.0239	.0071	
Croft	.1743	.1038		.0287	.0128	
Erabu	.1842	.0895	1.21	.0282	.0157	49.50‡
Estate	.1594	.1091		.0216	.0168	
Mean	.1697	.0973		.0256	.0131	

*Estimated regression line at height $r = 40$.
†Estimated regression line slope.
‡Significant at one percent level.

varieties exhibited substantially the same variation (Table III), and bridge distribution per cell was substantially the same for each variety (Table V).

It has been pointed out by Sparrow[12] that species differences in number and size of chromosomes can generally be correlated with differences in the reaction to radiation. It is apparent that these factors do not apply in the case at hand, since the chromosomes of all lilies used are about the same length and all have the same chromosome number.

A possible source of differential sensitivity might lie in structural abnormalities in as much as radiation was applied to synapsed chromosomes. The "Croft" variety of *L. longiflorum* is heterozygous for a translocation while "Ace," "Estate" and "Erabu" have 12 pairs of normal homologues. Within the sensitivity of the tests used, no significant differences were noted between the radiation reaction of "Croft" and the three "normal" varieties. While this does not rule out the possibility that under some conditions such alterations may be a source of variation, no evidence of such an effect was found in this work.

The fact that previous workers have shown that variations in radiation response can be due solely to genetic factors is more pertinent to our understanding of data presented here. It is of interest to look at the origin of the species tested. "Regale" is an ancient species of *Lilium — L. regale.* It is propagated both by vegetative division and by seeds. Although the origin of the plants tested here is not known, it is possible that they represent a clone; on the other hand, if they were propagated from seed, it is probable, because of current nursery practice, that they represent a highly inbred, relatively homozygous population.

All plants of "Creelman" belong to a single clone, vegetatively propagated from a single selection from an F_1 hybrid between *L. regale* and *L. sargentiae* (a species closely allied to *L. regale*[14]), and although undoubtedly more or less heterozygous, are genetically uniform. Thus Group 2 varieties are related to each other and each is highly uniform genetically.

TABLE V. Homogeneity chi-square test for four varieties of *L. longiflorum* at three doses of irradiation in Experiment 2

Varieties		Bridges per cell at pachytene				Bridges per cell at diakinesis				
		0	1	2	3	0	1	2	3	4
					20r					
Ace	Ob.	470	20			753	50	6	1	
	Ex.*	482.0	8.0			756.1	47.4	5.5	1.0	
Croft	Ob.	268	2			290	16	3	0	
	Ex.	265.6	4.4			288.4	18.1	2.1	0.4	
Erabu	Ob.	557	2			301	20	2	1	
	Ex.	549.8	9.2			302.4	19.0	2.2	0.4	
Estate	Ob.	204	1			171	9	0	0	
	Ex.	201.6	3.4			168.0	10.5	1.2	0.2	
		$\chi^2(3) = 26.83$†				$\chi^2(9) = 3.93$				
					40r					
Ace	Ob.	441	49	9		487	52	13	1	0
	Ex.	454.1	39.4	5.5		478.7	55.8	16.9	1.3	0.3
Croft	Ob.	312	23	1		369	45	13	1	0
	Ex.	305.8	26.5	3.7		370.5	43.2	13.1	1.0	0.3
Erabu	Ob.	293	24	2		382	53	17	1	1
	Ex.	290.3	25.2	3.5		393.0	45.8	13.9	1.1	0.3
Estate	Ob.	200	12	3		264	25	10	1	0
	Ex.	195.7	17.0	2.4		259.7	30.3	9.2	0.7	0.2
		$\chi^2(6) = 10.00$				$\chi^2(12) = 7.65$				
					60r					
Ace	Ob.	355	46	13	1	377	60	21	4	1
	Ex.	363.4	40.8	10.1	0.7	373.9	60.8	24.2	3.3	0.8
Croft	Ob.	235	25	5	1	231	36	17	2	1
	Ex.	232.9	26.2	6.5	0.4	231.8	37.7	15.0	2.1	0.5
Erabu	Ob.	308	33	6	0	146	27	9	1	0
	Ex.	303.9	34.1	8.5	0.6	147.8	24.0	9.5	1.3	0.3
Estate	Ob.	215	21	7	0	144	23	11	1	0
	Ex.	212.8	23.9	5.9	0.4	144.6	23.5	9.3	1.3	0.3
		$\chi^2(9) = 5.13$				$\chi^2(12) = 2.93$				

*Ex = expected value on the basis of homogeneity.
†Significant at one percent level.

In contrast, both varieties in Group 1 arose from a complicated series of hybrid crosses involving a diploid form of *L. tigrinum, L. bulbiferum, L. dauricum,* and *L. concolor.* "Enchantment" is a specific selection, clonally propagated, and should be highly uniform though relatively heterozygous. "Midcentury" on the other hand is a mixture of seedlings from this same cross. Thus it should be as heterozygous as "Creelman" but much less uniform.

It is then not surprising to find a difference in sensitivity between the two groups. The related forms within each group must share certain physiological or biochemical features which account for their similarity to reaction to radiation. Similar reasoning can account for the differences between groups and similarities within groups of the distribution of bridge frequency per cell.

It is tempting to speculate that the hybrid nature of the forms in Group 1 may in some way contribute to the increased sensitivity, greater variability and other differences in reaction between the two groups. The idea that heterozygosity or its related heterosis may possibly be at the root of these differences has its attractions, since the parents of "Creelman" (*L. regale* × *L. sargentiae*) show a close affinity and "Regale" is most certainly the most homozygous of all the varieties involved here. In the same connection it should be noted that "Creelman" is about 50 percent more sensitive than "Regale."

The greater variability of distribution of bridge frequency per cell in Group 1 compared to Group 2 is difficult to understand since "Enchantment," which should be highly uniform genetically, showed this variation nearly as much as "Midcentury" which is non-uniform. It may be that there is a physiological basis for this variation which is not present in Group 2 forms.

The cytology of two groups showed that meiosis in Group 1 varieties is slightly irregular with occasional univalents appearing at diakinesis, and we have no such evidence of irregularity in the Group 2 varieties. There is a possibility that this in some way is related to the observed differences in reaction between these groups.

The uniformity of the reaction to radiation of the several varieties of *L. longiflorum* indicates that no gross genetic differences in this respect exist between these varieties, and supports the general observation made by most workers that closely related plants in general show the same radiation sensitivity.

Summary

Sporocytes of eight different forms of *Lilium,* including four horticultural varieties of *L. longiflorum,* at known stages of meiotic prophase were subjected to Cobalt-60 gamma irradiation. Cytological aberrations were scored at anaphase I.

Variations in sensitivity between the several varieties and species irradiated indicated that closely related forms had similar sensitivities and that more distant relationships were accompanied by differences in net reaction. Hybrids between distant species tended to be more sensitive and showed greater variation than the relatively homozygous and highly inbred pure species.

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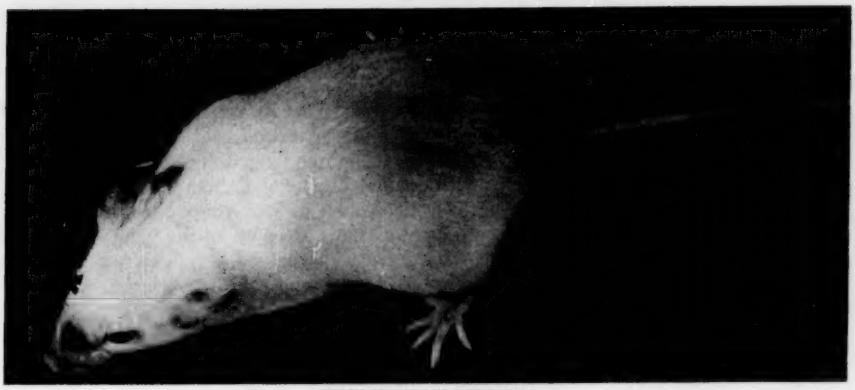
HIMALAYAN, A NEW ALLELE OF ALBINO IN THE MOUSE

MARGARET C. GREEN*



HIMALAYAN MOUSE

Figure 6

An eight-month-old female himalayan mouse ($c^h c^h aaB\text{-}DD$) showing light body fur and dark nose, ears, and tail.

MUTANT genes which cause light or white body fur and dark extremities are common in mammals. The Himalayan rabbit[1], Siamese cat[4], "albino" guinea-pig[8], and "partial albino" hamster[6] are of this type. In all these cases the mutation appears to be one of an allelic series similar to the albino series in mice. It is remarkable, therefore, that until very recently no similar mutation has been known in the house mouse, an animal which has been very extensively bred in captivity. Mohr[5] in 1939 described a mouse with white body and dark extremities caught in the wild on a German island, but gave no genetic data on the character. Dickie[2] described a single mouse heterozygous for albino, *c*, which was normal wild-type until about five months of age, when it slowly began to turn white except on its nose, ears, and tail. After several months it showed the classical himalayan phenotype but failed to transmit the character to its descendants.

In October, 1958, at the Roscoe B. Jackson Memorial Laboratory, in a cross of DBA/2J (non-agouti brown dilute, *aabbdd*) with AKR/J (non-agouti albino, *aacc*) a single white male mouse was found in a litter of seven by Mrs. Madeline Jewett. It was thought to be a mutation to albino and was set out for testing with an albino female. A few weeks later its nose, ears, and tail had become dark, and it was immediately recognized as a possible occurrence of the long expected himalayan mutation. Subsequent crosses showed this to be the case, and the gene was therefore named himalayan and given the symbol c^h.

Genetics

The original male was mated to several AKR females. These matings produced only albinos and mice like the original male, thus confirming the hypothesis of allelism of the new mutant and *c*. Matings between mice like the original male, presumed to be $c^h c \times c^h c$,

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produced a third type, darker than the parents, and presumed to be $c^h c^h$. Matings of various types were made among the offspring of these crosses, as well as outcrosses to C57BL/10 (*aa*). The segregation of c^h in all these crosses is given in Table I. All crosses give good agreement with expectation on the assumption of single factor segregation except cross 5 for which the deviation from expectation is highly significant. There is no obvious explanation for this deviation other than chance, and since all other evidence favors the single factor hypothesis, we may conclude that the himalayan character is caused by a single gene at the albino locus.

TABLE I. Results of matings with himalayan, c^h

Cross	Parents	Offspring			
		$c^h c^h$	$c^h c$	cc	Total
1	$c^h c \times cc$	—	47	47	94
2	$c^h c \times c^h c$	39	83	47	169
3	$c^h c^h \times c^h c$	40	43	—	83
4	$c^h c^h \times c^h c^h$	60	—	—	60
		CC	Cc^h	$c^h c^h$	
5	$Cc^h \times c^h c^h$	—	19	44	63
6	$Cc^h \times Cc^h$	55		15	70

Description

The mice on which the following descriptions are based were offspring of the above crosses and were all non-agouti. Some matings may have been segregating for *b* and *d*, but most of the mice observed were probably carrying at least one *B* and one *D* allele. Of seven mice tested, six were *B-D-* and one was *bbDD*.

Mice homozygous for c^h are indistinguishable from albinos at birth. At about one week of age they are slightly darker than albinos, particularly on the tail. The juvenile coat, when fully grown, is pale tan with little evidence of darkening of the extremities except on the tail. The body color may be uniform or may be slightly lighter across the shoulders and darker toward the tail. At the first molt the nose, ears, tail, and scrotum, but not the feet, become considerably darker, and the rest of the body becomes lighter. The dark hair on the nose extends back to about the anterior border of the eyes. The body fur, both dorsal and ventral, is lightest on the anterior half of the body and darkens gradually from the middle of the body back to the tail. There is often a particularly dark ring of body fur next to the tail and the skin of the scrotum may be very dark. The ears are darkest on their anterior border. The feet never become dark as they do in the Himalayan rabbit.

At subsequent molts the body may become somewhat lighter and the extremities darker, but there is considerable variation. Because of the genetic heterogeneity of the stock it was not possible to decide whether the variation was due to genetic or environmental causes, but it seems likely that at least some of it was due to variations in temperature. A preliminary experiment indicated that in $c^h c^h$ mice, as in Himalayan rabbits[7] and Siamese cats[3], development of pigment is temperature sensitive, and is promoted by low temperature. Under normal laboratory conditions with temperatures ranging between 70° and 78° F., the hair on the nose, which is the darkest part of $c^h c^h$ mice, is not as dark as the nose hair of *C-* mice of otherwise comparable genotype.

The eyes of $c^h c^h$ mice are not pigmented at birth but become darker with age, and are ruby colored at weaning.

Figure 6 shows an eight-month-old female of the genotype $c^h c^h aaB\text{-}DD$.

Himalayan mice heterozygous for *c* ($c^h c$) are indistinguishable from $c^h c^h$ and *cc* at birth but intermediate between the two at about one week of age. Their juvenile coats are very pale buff, hardly distinguishable from white. At the first molt the extremities darken but never become as dark as in the homozygotes. The eyes are intermediate between those of homozygotes and albinos.

The nose of one $c^h c^h$ tested mouse known to be *aabbDD* was quite brown compared to the noses of the $c^h c^h aaB\text{-}D\text{-}$ mice which were much blacker. It is not known to what extent other color genotypes would be recognizable in the pres-

ence of $c^h c^h$. In any case they would probably not be distinguishable until after the first molt.

To determine the effect of c^h in combination with other alleles of the albino series, crosses were made with extreme dilution, c^e, and chinchilla, c^{ch}. The $c^e c^e$ mice were from the c^e inbred strain which is wild-type at other color loci, and the offspring were therefore $c^h c^e AaB\text{-}D\text{-}$. They were dark eyed from birth with a pale juvenile coat. At the first molt the ears, nose, and tail darkened, with the result that in the adult the extremities were darker than in $c^e c^e$ but lighter than in $c^h c^h$ and the body was lighter than in $c^e c^e$ but darker than in $c^h c^h$.

A cross to chinchilla was made using a himalayan parent known to be *aaBBDD*, and a chinchilla parent from the SEC/1 strain, $c^{ch} c^{ch} aabb$. The offspring were therefore $c^{ch} c^h aaBb$. Homozygous chinchilla animals of this genetic constitution at the *a* and *b* loci are a dull black or sepia. Heterozygotes with c^h are very much lighter, a light golden brown. Their noses and tails darken at the first molt but are never as dark as in $c^{ch} c^{ch}$.

Heterozygotes of genotype Cc^h are indistinguishable from *CC*.

The new mutant allele, like its predecessors at this locus, is thus fully recessive to wild-type, but forms intermediates with all the lower alleles in the series. In depth of color produced, it is below c^{ch} in the series; it is above c^e in color of the extremities but below it in color of the body.

Summary

Himalayan, c^h, a new allele at the albino locus in the house mouse, is described. Homozygous himalayan mice have a dark nose, ears, and tail, and a light but not white body. The gene is recessive to wild-type, *C*, but forms intermediates with the other alleles in the series, c^{ch}, c^e, and c.

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EXPRESSIVITY OF A MIDGET MUTANT IN DROSOPHILA PERSIMILIS

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THE extent to which environmental factors can modify the expression of an organism's genotype depends upon the *norm of reaction* of that genotype. Greater variability which most mutants display throughout a range of environments usually contrasts sharply with the uniformity of reaction expressed by the "wild type" throughout the same range. Schmalhausen[10] described such reactivity of mutants as morphoses in the organism: "All really new reactions of the organism are never adaptive. They arise together with mutations as distinct expressions of the latter in different environments. . . . Morphoses [new reactions including new mutations] are *very unstable*. . . . All reactions of living matter, which are also based upon mutations, become adaptive only in the course of their historic development in certain environments."

A spontaneous mutant displaying great variability in stock culture appeared in 1955 in one of our stocks of *Drosophila persimilis* descended from a single wild female (WW38) collected in 1953 at the White Wolf locality in Yosemite National Park[12]. It appeared as a small body size, or midget, in a large proportion of flies from every generation; but on inbreeding, the progeny were always highly variable in body size. In very crowded cultures not only were some flies extremely tiny (less than half normal size) but the proportion of small flies was much greater than in less crowded cultures. Nevertheless, the range in size always included some normal-sized flies. In addition to reduced body size, an imbalance in the sex ratio was always apparent: males always outnumbered females significantly. Midget females also had a low egg-laying capacity.

Preliminary examination raised the question of the role of the environment in the expression of the midget. The increase in number of midget flies from crowded larval conditions could be due to insufficient nutrition as a limiting factor in the expression of midget. Among genetic mechanisms controlling variable expression could be incomplete penetrance of a point mutation, the segregation of modifiers affecting expressivity of the point mutation, or recombination of a polygenic complex affecting body size. Examination of hybrid (midget/wild) larval salivary chromosomes revealed no sign of chromosomal aberrations.

Four general series of crosses were made to elucidate these phenomena: first, progeny of midget flies and progeny of wild type were grown separately in varying densities on Spassky[11] cream-of-wheat-molasses (COWM) or on Kalmus[6] medium; second, with a knowledge of the range of variability, crosses between wild type and midget were made for the mode of inheritance; third, one generation of selection for increase and decrease in body size was made to check on the existence of modifiers; and fourth, crosses to markers on the autosomes determined the chromosomal location of the main effect.

Materials and Methods

For egg collecting, flies were mated in half-pint bottles containing plastic spoons filled with yeasted food and lampblack. Spoons were changed daily; eggs removed from spoons in groups of 10 on a needle were transferred to the surface of slanted food in 9.5 x 2.5 cm. vials.

All laboratory strains and the earlier tests were made on COWM[11], but later

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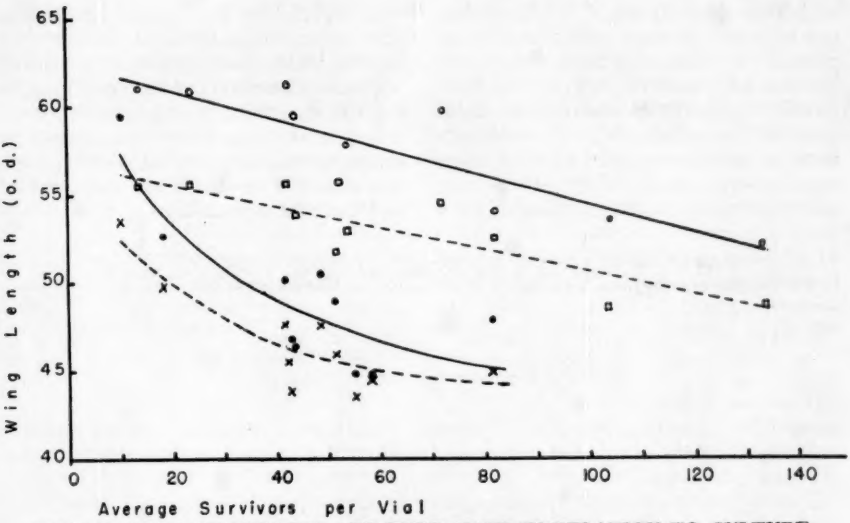

WING LENGTH OF MIDGETS AND WILD TYPE IN RELATION TO CULTURE DENSITY

Figure 7

Observed relationships between average number of survivors per culture (COWM) and wing size: X = midget ♂♂, black dots = midget ♀♀, open squares = wild type ♂♂, open dots = wild type ♀♀. Fitted curves: ♂♂ dashed lines, ♀♀ solid lines.

tests were made on Kalmus[6] medium. For vials containing Kalmus medium, 4 cc. were cooled in a slanted position, and prior to egg planting 14 mg. of bakers' yeast in 0.1 cc. of water was added plus a small strip of paper towelling. A drop or two of distilled water was added to Kalmus medium periodically if the desiccation of the agar seemed too great. All tests were done at 25° C.

Since the exact body size is difficult to measure accurately and since wing length is highly correlated with body size, wing length was used for size (distance from the apical bristle at the distal break of the costal vein to the joining of the third longitudinal vein with the costal vein). An ocular micrometer (calibrated at 31 divisions per mm.) was used to measure wing length and all references in the following are given in ocular divisions (o.d.).

The experimental strains of *D. persimilis* used were the WW38 (midget) and WW13 (normal size) collected in 1953 by Spiess. Both strains were homokaryotic for the Whitney arrangement of chromosome 3. For linkage determinations, the marker strains, including Delta cardinal (2), orange Blade Scute purple (3) and Curly (4), were obtained from the laboratory of Professor Th. Dobzhansky. All matings consisted of 10 flies of each sex per mating bottle.

Results

A. Variability of size with density of culture

Randomly selected individuals from the midget strain were mated, and eggs were planted at increasing densities of 50 up to 500 in vials containing COWM (three vials per density). Parallel wild-type density vials were made in the same manner for comparison. Table I and Figure 7 illustrate the principal effects: (1) at low densities midget and wild type are nearly identical in size and variability; (2) the midget becomes expressed in logarithmic inverse proportion to the number of survivors (density) per culture (midget female on survivors: $\log Y = 1.94 - (0.122) \log X$; and midget males on survivors: log Y

$= 1.86 - (0.092) \log X$); (3) as density increases average wild-type size decreases in linear proportion (wild females on survivors: $Y = 62.5 - (0.027) X$; and wild males on survivors: $Y = 56.9 - (0.023) X$); (4) total survival is greater for wild type at every egg density except one; (5) midget males outnumber midget females by a ratio of 1.5:1 or 2:1 in the higher densities where the body size is smaller; (6) midget males had consistently greater uniformity than midget females throughout the experiment; and (7) wild-type flies are more uniform than midget in comparable densities of survivors (average standard deviation for midget 3.82 and for wild type 2.80).

TABLE I. Wing size and survival of midget and wild-type flies grown on COWM

Egg density*	Sex	Midget			Wild type		
		N*	M*	σ*	N*	M*	σ*
50	♀♀	17	59.5	2.42	19	61.1	1.25
	♂♂	15	53.2	2.36	20	55.5	2.54
100	♀♀	29	52.8	3.13	39	60.9	1.69
	♂♂	24	49.8	1.99	33	55.4	1.60
150	♀♀	64	50.2	4.01	57	59.3	2.42
	♂♂	61	47.6	2.77	72	53.8	2.25
200	♀♀	52	46.7	4.38	71	55.9	3.27
	♂♂	75	45.2	3.74	83	51.6	2.41
250	♀♀	72	50.4	4.47	75	57.8	2.56
	♂♂	74	47.4	3.20	86	52.9	1.93
300	♀♀	71	44.8	4.45	134	53.7	6.18
	♂♂	101	44.8	3.87	113	52.1	4.45
350	♀♀	104	47.8	6.42	61	61.1	1.56
	♂♂	144	44.9	3.73	63	55.6	1.34
400	♀♀	55	44.5	3.06	125	59.4	1.64
	♂♂	109	43.2	2.82	92	54.2	1.77
450	♀♀	66	48.9	6.51	208	52.1	3.47
	♂♂	86	45.6	4.66	195	48.0	4.29
500	♀♀	53	46.2	4.58	169	53.1	4.76
	♂♂	75	43.7	3.83	141	48.1	4.67

*Each egg density was done in triplicate. N = total number adults eclosing, M = mean wing length in ocular divisions (31 divisions per mm.), σ = standard deviation.

TABLE II. Wing size and survival of midget and wild-type flies grown on Kalmus medium

Egg density*	Sex	Midget			Wild type		
		N*	M*	σ*	N*	M*	σ*
10	♀♀	3	48.7	6.41	5	61.8	3.35
	♂♂	5	46.0	5.43	2	57.0	0
20	♀♀	15	53.4	3.78	5	63.6	0.87
	♂♂	15	49.7	2.69	1	57.0	0
30	♀♀	28	52.2	4.28	8	63.4	0.93
	♂♂	25	49.9	2.60	4	57.0	0.82
40	♀♀	20	54.5	4.70	20	61.8	2.55
	♂♂	35	49.7	2.94	14	56.6	1.33
50	♀♀	17	51.2	3.83	19	61.6	3.16
	♂♂	29	47.2	3.08	17	57.5	1.61
80	♀♀	33	51.1	3.64	29	58.3	4.72
	♂♂	56	47.9	2.11	45	54.0	3.97
Average	♀♀		51.85			61.75	
	♂♂		48.40			56.52	

*See Table I.

Because midget and wild were not separable at low egg densities, it was laborious to attempt further crosses by planting large numbers of eggs. In addition, a density effect on the expression of midget suggests a nutritional basis. Consequently the low-nutrition Kalmus medium was tried with low egg densities in the hope that midget and wild type could be distinguished without the labor of transplanting hundreds of eggs. Data from Kalmus medium tests are given in Table II. Egg densities were made in triplicate, increasing by 10 up to 50 and 80 eggs per vial. Clearly the midget and wild type express nearly constant differences throughout these low densities, although the wild type decreases slightly in size at the highest density. Kalmus medium then provided conditions for expression of midget at much lower egg densities than those necessary for complete expression on COWM. Several other interesting comparisons may be made between results on COWM and on Kalmus medium: (1) total survivors per culture are less for wild type than for midget, a reversal with respect to survival on COWM; (2) midget males are in excess of females only in the higher densities; (3) males have smaller standard deviations than females both in wild type and midget; and (4) wild type was more uniform in all densities except the highest.

B. Selection for change in midget body size

Although the midget stock had been inbred for some time, there was a possibility that the high variance in its expression could be due in part to segregation of modifiers. Four sets of selection crosses within the midget stock were made for one generation to test for modifier heterogeneity. Parent flies of those selected were grown on Kalmus medium in vials, the largest and smallest progeny then selected in four sets (large male × large female, large male × small female, small male × large female, and small male × small female) and mated at 15 days of age in half-pint bottles provided with spoons of COWM. Eggs were collected and planted in densities of 80 per vial on Kalmus medium. Results are given in Table III.

Obviously size variation within the midget stock is not due to modifiers to any measurable extent. Smallest parents produced largest daughters; however, male progeny showed no significant differences between selected parent sizes. There is no significant association be-

TABLE IV. Wing size and survival of F_1 flies (midget/wild) grown on Kalmus*

Wing size	F_1 (M♀♀ × +♂♂)		F_1 (+♀♀ × M♂♂)	
	♀♀	♂♂	♀♀	♂♂
37-39	0	0	0	0
40-42	0	2	0	3
43-45	4	4	3	10
46-48	3	9	1	12
49-51	4	11	2	15
52-54	5	11	5	23
55-57	15	1	20	5
58-60	11	0	27	0
61-63	9	0	13	0
64-66	0	0	0	0
Average size	55.5	49.3	57.3	49.9
% Survival	20.4	15.2	28.5	27.2

*Fifty eggs per vial on five vials in each cross.

TABLE III. Results of selection for large and small size from midget stock raised on Kalmus medium

Cross	Sex	N	Survival %*	M	σ
1. Small × small	♀♀	21		55.6	2.50
♀♀ (46.8 av.) × ♂♂ (44.5 av.)	♂♂	44	20.3	50.1	2.64
2. Large × small	♀♀	72		52.9	4.59
♀♀ (56.8 av.) × ♂♂ (45.0 av.)	♂♂	68	29.1	50.1	2.79
3. Small × large	♀♀	42		54.7	3.15
♀♀ (47.4 av.) × ♂♂ (52.7 av.)	♂♂	47	22.3	48.4	3.25
4. Large × large	♀♀	30		53.4	4.06
♀♀ (57.0 av.) × ♂♂ (52.7 av.)	♂♂	35	27.1	49.9	3.84

*Vials contained 80 eggs each. From three to six vials were used per cross.

tween density of culture and size of midgets; consequently, the variation in size within the midget stock seems indeterminate at this time, a situation which is to be expected with mutants, as Schmalhausen points out above.

Curiously enough, the sex ratio was disturbed significantly only in the cross in which both parents were small. This result answers the question of whether excess of males is due to excess male zygotes or better survival of males in culture. Certainly the fact that the sex ratio is not disturbed either in very low densities (Table II) or if one or both parents approach normal size tends to favor the differential survival hypothesis. Fecundity in both sexes is greater for midget flies of nearly normal size, but it is not clear what connection that fact may have with the change in relative survival of preadult stages. There is no significant association between sex ratio and density of culture in this experiment.

On the other hand there is a slight but significant maternal effect on survival percentage: depending on the size of the female parent, the average change is between five and nine percent. Larger midget females not only lay more eggs but their progeny are slightly more viable than those of smaller midget females.

C. Genetics of midget

To determine the mode of inheritance and variability of heterozygotes, reciprocal matings were made between midget and wild type. Five vials of each cross were made with a density of 50 eggs planted on Kalmus medium. Allowing for the density of the culture, an approximate size can be estimated for the sake of comparison, although the male data are far more reliable since they are more constant from vial to vial and test to test. Using the data from Tables II and III, one can estimate the following average sizes: midget females 53.3, midget males 49.3. Wild-type sizes are taken from highest density in Kalmus: wild females 58.3, wild males 54.0.

In Table IV it can be seen that for males midget size is dominant but for females it is incompletely dominant with a slight maternal effect. (Difference between reciprocal F_1 females is 1.8, $t = 2.11$, d.f. $= 120$, and $p = 0.04$.) In future tests male data are far more useful than female in making size comparisons. Incidentally, viability is again affected by the size of the mother.

Identification of midget with a linkage group was done as follows: before the details of wing measurement had been worked out, midget flies were crossed out to markers on chromosomes 2 and 3 (Delta and Blade Scute, respectively). F_1 males heterozygous for the marker and midget were mated to normal-sized females. The data clearly showed independence in each case.

TABLE V. Wing size and survival of backcross and test-cross progeny grown on Kalmus*

		M♂♂ / + × + / ♀♀		Non-Curly in cross: Cy♂♂ / Midget × +♀♀ / +		
Wing size	♀♀	♂♂	♂♂%	♀♀	♂♂	♂♂%
42-43	0	2	1.7	0		
44-45	1	7	6.1	0	3	8.1
46-47	2	10	8.7	1	1	2.7
48-49	9	22	19.1	0	9	24.3
50-51	4	29	25.2	2	14	37.8
52-53	8	30	26.1	2	8	21.6
54-55	20	15	13.1	10	2	5.4
56-57	20	0		23	0	
58-59	22	0		11	0	
60-61	5	0		2	0	
Av. wing size	55.7	51.9		56.2	50.1	
% Survival	21.4	23.0		17.6		

*Fifty eggs per vial on 10 vials in each cross.

Wild female × midget/Delta male produced 40 Delta midget, 31 Delta normal, 50 wild midget, and 30 wild normal.

Wild female × midget/Blade male produced 27 Blade midget, 4 Blade normal, 32 wild midget, and 16 wild normal.

Backcrossing of heterozygote midget/wild F_1 males to wild females produced the data given in the four left-hand columns of Table V. There are no distinct size classes, although the flattening of distribution suggests a bimodality and segregation of M and + classes. Linkage to chromosome 4 is suggested by the following test: F_1 males (Curly/midget) were tested to wild-type females. The data appear in the three right-hand columns of Table V. Curly wings are badly distorted with blisters so that measuring them is not helpful in establishing independence between midget and Curly. If independence was the case, about the same percent survival of normal-sized flies should occur among non-Curly flies as in the backcross. For non-Curly males the mean is not significantly different from the expected mean of 49.9 for M/+. Apparently there are no +/+ males. It seems likely on the basis of these male data that Curly has segregated from midget and that the latter is located on chromosome 4.

Discussion

Dwarf mutants are common in wild populations of both *D. pseudoobscura* and *D. persimilis*[5], but their detection and classification are difficult owing to their greater variability and alterability by environmental factors. The midget reported here likewise is highly variable and would go undetected in low-density cultures. Since this mutant was discovered in our stocks about 15 generations after extraction from the wild population, it is not possible to say whether the mutation occurred in nature or in the laboratory; it would easily have gone undetected owing to the fact that females heterozygous for midget are nearly normal in size.

Expression of midget is marked by nutritional deficiency, as borne out by high-density effects on cream-of-wheat-molasses food and by consistent small size in Kalmus medium. While wild type decreases also with increasing density of larvae due to diminishing availability of yeast[7], Kalmus medium plus yeast is sufficient for normal body size of wild-type flies at densities up to 50 eggs per culture (about 12 survivors per culture). Obviously, then, midget expression cannot be dependent on yeast availability alone, and omission of some other factors in Kalmus medium must be considered. Kalmus medium contains no major source of amino acids, although it does contain a source of nitrogen ($(NH_4)_2\ SO_4$). A principal ion missing is Na^+ in Kalmus medium, which may be a principal factor in a lowering of survival for wild type and/or bringing out complete expression of midget size. Also, Kalmus medium has an acid pH (about 3.7) since it contains tartaric acid to lower the growth of bacteria. Either the sodium deficiency or the acidity or both might affect differential survival and expression of midget.

From the ineffectiveness of a single generation of selection within the midget stock it is apparent that modifiers are not segregating and that the variability of the mutant is largely attributable to environmental factors. From the evidence of crosses using genetic markers, midget would seem to be dominant in males and semidominant in females located on chromosome 4. It has had little or no adaptational history in the wild population and consequently acts as a morphosis, or unstable new reaction, of the organism, in the sense of Schmalhausen. It is tempting to postulate that better survival of midgets than wild type in Kalmus medium may be the result of economy of nutrition because of small body size.

The sex ratio effect of the mutant is even more difficult to define or ascribe to known factors. Males are present in overabundance whenever both parents are midget and very small and when culture conditions for developing larvae are crowded. It would seem likely that midget male zygotes are equal to female zygotes in number but that they have superior survival value in dense cultures. The effect of parent size upon the sex ratio, however, is far from clear.

Summary

A small body size (midget) mutant with a sex ratio effect favoring males and located on chromosome 4 of *D. persimilis* is described. Its expression when cultured in conventional fly food is logarithmically related to the density of the culture, in contrast with wild type which decreases in size in linear proportion with increasing density. At low densities midget flies are indistinguishable from normal size, while at high densities the average midget fly is about 0.25 mm. shorter; but the range in size always includes a few normal-sized flies. Culturing midget and wild-type flies on Kalmus medium (of pH 3.7, low in sodium salts, and limiting in yeast growth) permits a low-density population to produce consistent differences between average midget and wild type, although size ranges still overlap slightly. That higher variability of midget

is not due to segregation of modifiers was demonstrated by an attempt to select for large and small size within the midget stock, with ineffectual results. While the mutation is dominant in males and partially dominant in females, its high instability must be due to incomplete penetrance. Although this variability is indeterminate, larger-sized midget females produce more viable cultures than small-sized female midgets. Finally, a better survival of midgets than of wild-type flies in Kalmus medium may be a result of the economy of nutrition because of small body size.

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Principles of Human Genetics

(*Continued from page 66*)

presentation. A further improvement is the use of a number of new photographs which more clearly picture several of the genetic characters previously described. In addition, the publisher has so utilized type, format and paper that while significantly expanding the actual text he has essentially retained the book size of the first edition. The volume's attractiveness is increased at the same time.

Genetic diversity and standard breeding approaches are largely uncontrollable in man. Yet, "the human population is large, and its millions of unions give the geneticist an immense amount of material from which to select what he needs." The collection of data from families with appropriate family histories, the devising of special methods for giving long range views of information from one or two generations, the pooling of similar data for many families, etc., provide statistical possibilities for providing answers to many inheritance problems in man. Using these and other approaches, the text presents material for a well-rounded course in fundamental genetics. Along with, and following, a careful development of basic genetic tenets are a number of chapters dealing accurately and interestingly with various phases of eugenics. Chapter titles such as "Genetic Counseling," "Blood Groups," "Medicolegal Applications of Genetics," "Prenatal Interactions" and "Consanguinity" are indicative of some subjects covered. These lead to consideration of "Genetic Aspects of Race" and "Race Mixture" and, finally, "The Origin of Human Diversity." References at the end of each chapter indicate the basic information sources, as well as where further details may be found. Separate author and subject indexes are provided.

The materials and illustrations are based on man—one of the most difficult of genetic materials. This factor makes the book of special interest. It is knowledge of the genetics of man which is of primary, ultimate interest to the geneticist and to scientists in several other fields, as well as to many beginning and lay students.

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GENETICS OF X-RAY INDUCED PIGMENTATION IN JUTE

M. S. SARMA AND K. GHOSH*

CHANGES in pigmentation pattern resulting from irradiation have been reported by Granhall[5] in pears, Bishop[1] in apples, Richter and Singleton[9] and Sagawa and Mehlquist[10] in carnations, and Hough and Weaver[6] in peaches. Among the fruit trees the variations are of fruit color, and among carnations, of flower color.

Gamma irradiation of *Antirrhinum, Petunia, Lilium* and *Tradescantia* results in production of flowers bearing spots of changed color representing somatic mutations. In *Petunia,* a somatic mutation was obtained which involved a whole branch bearing white instead of the usual dark flowers[2].

Detailed studies on the nature of changes in pigmentation have not been made on the variants among fruit trees and many of the flower types. Mehlquist and Geissman[7] have shown that carnation is a periclinal chimera in which the outer layers are genetically different from the inner ones. Anatomical studies by Sagawa and Mehlquist[10] on radiation-induced flower color mutants in carnation have revealed that the mutation is a result of destruction of outer layers of cells by irradiation followed by regeneration of the tissue from endogenous layers. As the inner layers are genetically different, a new pattern of flower color results. Thus, at least in carnations, the variation in pigmentation is held to be a result of morphological alteration of the shoot apex and is not due to mutation.

The present report deals with a transmittible change in the pigmentation pattern induced by X-rays in *Corchorus capsularis.*

Material

Anthocyanin factors in C. capsularis

The *C,A,R* factors in *C. capsularis* control the pattern of anthocyanin pigmentation. *C* is a chromogen factor, essential for the production of color. Forms of *A* are known to occur as a series of multiple alleles (a-A-A^L-A^R-A^D), associated with the intensity and distribution of pigment. *R* is a pigment-reducing factor, the effect of which is most marked on the stem[3,4,8]. In the presence of *C* and one of the multiple alleles of *A*, *R* in a single (*Rr*) or double (*RR*) dose results in the production of green-pigmented types, while all red types are found to be associated with the homozygote, *rr*. Among all multiple alleles of *A*, except *a*, the intensity and distribution of pigment in the presence of *C* is discernible in the early stages of growth, but in *aa* the expression of pigment is delayed and is noticed only after the plants are four to five weeks old[4].

Origin of the pigmented mutant

Seeds of *Corchorus capsularis,* strain JRC-212, a full-green type having the genetic constitution *ccAARR* for the three anthocyanin factors, were treated in 1953 with various doses of soft X-rays, ranging from 1,000r units to 40,000r units. Then they were planted in the field with the untreated. In the X_1 generation, a number of normal looking plants and morphological variants were selected. When seeds from X_1 selections were grown, it was observed in the X_2 that progenies of a majority of the morphological variants were normal and thus similar to controls, indicating that most of the aberrations observed in the X_1 are either somatic or nontransmittible. However, seeds of one of the branches from a bifurcated but otherwise normal looking, full-green X_1 selection from the 45 K.V.P., 40,000r treatment segre-

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gated in the X_2 for pigmentation. The pigmentation pattern proved to be transmittible, and studies on the genetic relations of this pigmented variant are reported in the present paper.

Description of the pigmented mutant

The X-ray induced pigmented type, hereafter termed the pigmented mutant, is full-green and indistinguishable from the parental strain JRC-212 until it is about six to seven weeks old. At this stage, the lower portion of the stem develops coppery-red color which soon changes to dark coppery-red. The axillary buds are light pink but their bases are deeper colored. The stipule, which is light coppery-red in the six to seven week stage, changes later to pink-red. In pigmented types of normal *C. capsularis,* the ventral surface of the peeled bark shows fairly uniform pigmentation, but in the pigmented mutant, the ventral surface of bark exhibits intense pink color at the point of attachment of the axillary bud. With the onset of maturity, the general pattern and distribution of pigmentation are observed to be markedly different from those of the naturally occurring pigmented types; all parts of the plant, except the upper surface of leaf, are pigmented dark coppery-red with flushes of pink at the nodes. The lower portion of the stem shows dark pink. The most important diagnostic character is, however, the pink color of the integuments, noticed even when ovaries at flowering stage are dissected. The seed coat of normals, whether belonging to the full-green or pigmented type, is colorless until about two weeks after fertilization, when it changes first to light brown, then to dark brown and finally to a chocolate color at seed maturity. In the pigmented mutant, the seed coat, which in the very early stages is pink, changes progressively to dilute pink, red, red-brown and ultimately to a clotted-blood color at seed maturity. Seeds of the mutant average about half the size and weight of normal seeds. Although the embryo is normal, the endosperm is imperfectly developed; this results in seeds with extremely low capacity for germination. Consequently, the mutant has so far been maintained by growing the heterozygous normals.

Experimental Procedure

To determine the genetic relations of the pigmented mutant with the standard *C, A* and *R* alleles, it was crossed to the following tester strains:

	Pigment type	*Constitution*
JRC-212	full-green	*ccAARR*
D154	green-coppery-red	*CCAARR*
Maniksari	dark red	CCA^DA^Drr

The crosses were studied in the F_1, F_2 and backcross generations. The segregation in the X_2, in which the pigmented mutant was first observed, was also recorded.

Results

X_2 generation

The X_1 selection was full-green, and when its seed was planted, the resulting progeny were all scored as full-green and nonsegregating until the eighth week. After this stage, successive counts showed larger proportions of the pigmented mutant and a corresponding decrease in the number of full-greens, indicating that the expression of the phenotype is fully attained at a particular stage of growth and not earlier. The final scoring, at the early fruit stage, gave a 3:1 segregation consisting of 136 full-greens and 40 pigmented mutants.

This suggested a single factor difference between the two, the pigmented mutant type being recessive to full-green in the X_2. The factor is designated P_i (induced pigment). On the assumption that the full-green and pigmented mutant differ by this single factor alone, the pigmented mutant may be expected to be $ccAARRp_ip_i$ and the full-green segregant $ccAARRP_i/-$. Therefore, the untreated control and the normal strains may be presumed to carry P_i in addition to their normal complement for the *C, A* and *R* factors.

Crosses

1. JRC-212 × pigmented mutant. All of the F_1 plants were full-green. The F_2 segregated in the ratio of 3 full-

green: 1 pigmented mutant, as shown in Table I.

Backcrosses of the F_1 with both parents gave the following results:

$F_1 \times$ JRC-212—All six crosses yielding 130 backcross progeny gave only full-green plants.

$F_1 \times$ pigmented mutant segregated into full-green and pigmented mutant in a 1:1 ratio, as shown in Table II.

The simple dominance of full-green over pigmented mutant observed in this cross indicates that the recessive factor *c*, carried by the parental strain, has not been affected by X-ray treatment and that the element concerned in the production of the pigmented mutant phenotype is distinct from the *C* allele. Further, the mutant differs from the standard full-green tester by a single factor and the pigment appears to be expressed only when this factor is present as a homozygous recessive.

2. D154 $\times$ pigmented mutant.

All of the F_1 plants were green-coppery-red. The F_2 families segregated into green-coppery-red (like D154), pigmented mutant and full-green in a 9:4:3 ratio, as shown in Table III.

Backcrosses of the F_1 were obtained with both parents. Two families derived from the backcross with D154 and comprising 41 plants were all green-coppery-red and similar to D154. Six families, obtained from the backcross with the pigmented mutant, showed segregation into green-coppery-red, pigmented mutant and full-green in the ratio of 1:2:1 (Table IV).

Since the F_1 is green-coppery-red, a ratio of 12 green-coppery-red: 3 full-green: 1 pigmented mutant would have indicated simple dominance of *C* over *c* and of P_i over p_i. But the excellent fit obtained in the F_2 for a 9:3:4 ratio indicates that the expression of p_ip_i, the homozygous recessive, is obtained irrespective of whether *C* (dominant) is homozygous, heterozygous, or absent. The cross with green-coppery-red thus supports the results obtained in the cross with full-green to the extent that the action of p_ip_i is unconnected with the recessive *cc*. The testcross segregation in the case of $F_1 \times$ pigmented mutant is in accord with the interpretation that p_ip_i is completely epistatic in action over the *C* alleles.

The two crosses so far dealt with do not indicate, however, the relation of the new allele p_i to the alleles of *A* and *R*, since the irradiated type is *ccAARR*, and it appears from the data that *A* and *R* are both unaffected in the pigmented mutant.

3. Dark red $\times$ pigmented mutant. The F_1 plants were green-dark-red with marked reduction of pigment on

TABLE I. F_2 segregation in JRC-212 $\times$ pigmented mutant in two families

Classes	Observed	Ratio	Exp.	χ^2
Full-green	796	3	804.00	0.0796
Pigmented mutant	276	1	268.00	0.2388
Total	1072	4	1072.00	0.3184

P = 0.70 — 0.50

TABLE II. Backcross of the F_1 (JRC-212 $\times$ pigmented mutant) to pigmented mutant in five families

Classes	Observed	Ratio	Exp.	χ^2
Full-green	62	1	66	0.242
Pigmented mutant	70	1	66	0.242
Total	132	2	132	0.484

P = 0.50 — 0.30

TABLE III. F_2 segregation in D154 $\times$ pigmented mutant in four families

Classes	Observed	Ratio	Exp.	χ^2
Green-coppery-red	765	9	752.625	0.2035
Pigmented mutant	324	4	334.500	0.3296
Full-green	249	3	250.875	0.0140
Total	1338	16	1338.000	0.5471

P = 0.80 — 0.70

TABLE IV. Backcross of the F_1 (D154 $\times$ pigmented mutant) to pigmented mutant in five families

Classes	Observed	Ratio	Exp.	χ^2
Green-coppery-red	28	1	23.00	1.0870
Pigmented mutant	42	2	46.00	0.3478
Full-green	22	1	23.00	0.0435
Total	92	4	92.00	1.4783

P = 0.50 — 0.30

the stem as compared to the dark red parent. This supports the hypothesis that *R* is unaffected in the pigmented mutant, and as a result of the action of *R* carried by it, the F_1 falls under the green-red group rather than the red group which requires the presence of *rr*.

The F_2 segregated for all four factors. (Table V).

The F_1 was backcrossed to both dark red and pigmented mutant parents. The progeny of the testcross with the dark red parent segregated into dark red and green-dark-red, as shown in Table VI.

The data of this backcross indicate segregation only for *R* since the testcross parent carried *rr*. The backcross with the pigmented mutant gave the results shown in Table VII.

The F_2 segregation of this cross shows that 1/16 of the individuals fall under the pigmented mutant class. But in crosses with full-green (c*AR*) and green-coppery-red (*CAR*), both of which carry homozygous *R* in the present experiment, 1/4 of the F_2 progeny belong to the pigmented mutant type. If the constitutions of *C*, over which p_ip_i is epistatic, and the alleles of *A* are ignored for the moment, the dark red parent may be written as rrP_iP_i and the pigmented mutant as RRp_ip_i. In a cross between the two, 1/16 of the F_2 progeny will be expected to be each of rrP_iP_i and RRp_ip_i, if it is assumed that *R* and P_i are independently segregating factors. It is evident from the phenotype of the F_1 that the pigmented mutant used in this cross carries *RR*. It should be pointed out that homozygous *A*, as distinct from A^p, also occurs in similar frequency. As such, the presence of *AA* rather than *RR* may be considered essential for the action of p_ip_i. But in that case, coppery-red, which has the constitution *C/-AArr*, and green-coppery-red (*C/-AARR*) would not be recovered in the F_2, as *A* in this cross is carried only by the pigmented mutant parent and all segregants having *AA* would then have fallen phenotypically under the pigmented mutant class. As both coppery-red and green-coppery-red are obtained among the F_2 progeny, the data show that the action of p_ip_i is dependent upon the presence of homozygous *R* and that the alleles *A* and A^p are unable to influence the expression of p_ip_i.

TABLE V. F_2 segregation in dark red × pigmented mutant in two families

Classes	Observed	Ratio	Exp.	χ^2
Green-dark-red	126	99	124.910	0.0095
Full-green	70	60	75.703	0.4296
Dark red	49	36	45.422	0.2818
Green-coppery-red	37	33	41.637	0.5164
Pigmented mutant	26	16	20.187	1.6739
Coppery-red	15	12	15.141	0.0013
Total	323	256	323.000	2.9125

P = 0.70 — 0.50

Discussion

Experimental evidence presented above indicates that the X-ray induced change from P_i to p_i affects the *CAR* system of pigmentation patterns in *C. capsularis*. The action of p_ip_i in the presence of *RR* is also reflected in pigmentation of organs such as integuments, which are normally colorless, and in the extremely low viability of seed. Like almost all the mutations for qualitative characters induced by irradiation, p_i is a recessive mutation. However, induced mutations in organisms such as maize have been shown to be minute deficiencies which are associated with

TABLE VI. Backcross of the F_1 (dark red × pigmented mutant) to dark red in two families

Classes	Observed	Ratio	Exp.	χ^2
Green-dark-red	9	1	9.5	0.0263
Dark red	10	1	9.5	0.0263
Total	19	2	19.0	0.0526

P = 0.90 — 0.80

TABLE VII. Backcross of the F_1 (dark red × pigmented mutant) to pigmented mutant in two families

Classes	Observed	Ratio	Exp.	χ^2
Green-dark-red	6	3	6.375	0.0221
Green-coppery-red	5	3	6.375	0.2966
Pigmented mutant	11	4	8.500	0.7352
Full-green	12	6	12.750	0.0441
Total	34	16	34.000	1.0980

P = 0.80 — 0.70

the null effect of the parent gene. The deficiencies at the *A* locus in maize, *a-x*$_1$, *a-x*$_2$ and *a-x*$_3$, show phenotypic effects similar to *a* and are lethal in the homozygote. While *ax*$_1$ shows normal transmission in gametes, *a-x*$_2$ and *a-x*$_3$, which are probably larger deficiencies, are associated with appreciable reduction in transmission of gametes[11].

In the present experiments, the factor *pi* does not appear to affect the normal transmission of gametes. Support for this view is obtained from the F_2 and backcross segregations which yield pigmented mutant progeny in all three crosses. The mature embryo of seeds from *RRpipi* plants is normal in both selfed seeds and in those derived from crosses with *Pi*, but the endosperm of both types shows poor development; as a result, the germination of such seeds is greatly affected.

The allelism test of the pigmented mutant obtained in the cross with *CAR* (green-coppery-red) shows that *c* is not affected since full-green plants are found as normal segregants in both the F_2 and in the backcross with the mutant. If *c* had changed to a lower allele (recessive to normal *c*), the cross with *cAR* would still have shown 3 full-green : 1 pigmented mutant, but crosses with *CAR* and *CA*^{p}*r* would not yield full-green derivatives. Thus, it is apparent that *c* has not altered during treatment.

The recovery of green-coppery-red (*C/-AAR/-*) and coppery-red (*C/-AArr*) in the cross with *CA*^{p}*r* (dark red) rules out the possibility that *A* and *R* are involved in the change.

The evidence presented indicates that the change is restricted to a single locus, *Pi*, which has been altered to the recessive state, *p*$_i$. The normal functioning of *C*, described as a factor essential for the production of pigment[8], appears to be dependent on the existence of *P*$_i$. In the absence of *P*$_i$, the chromogen factor *C* is unable to act; consequently the alleles of *A*, which affect the intensity and distribution of anthocyanin pigmentation in the presence of *C*, fail to function. The *pi*-controlled anthocyanin pigment, on the other hand, has not been found to be affected by *A* and *A*^{p}, the two alleles of *A* tested so far. Thus, it is possible that the *C*-controlled anthocyanin is different in constitution from the *p*$_i$-controlled anthocyanin. Since *p*$_i$*p*$_i$ is epistatic over *C*, and the presence of at least one dose of *Pi* is essential for the normal action of *C*, the biochemical step for the synthesis of *C*-controlled anthocyanin would be placed after the *Pi* stage.

Previously, *R* was viewed as a factor which reduces the fully pigmented types to the corresponding green-pigmented state; a single dose of *R* is adequate for this action. In the present experiments the expression of *p*$_i$*p*$_i$ is obtained only when *R* is in a homozygous state. It appears, therefore, that *R* has a second function, i.e., the control of the action of *p*$_i$*p*$_i$. In this it behaves as a gene with dosage effect, two doses of *R* being necessary for the expression of *pipi*.

Summary

A full-green strain of *Corchorus capsularis* (JRC-212) having the constitution *ccAARR* for the anthocyanin factors was treated with X-rays. In the X_2 generation of a bifurcated but otherwise normal looking selection from the 45 K.V.P., 40,000r treatment, a pigmented type was obtained as a recessive segregant. The pigmented mutant is indistinguishable from full-green in the early stages; at later stages the stem is dark-coppery-red and the integuments of ovules are pink even at the flowering stage. Although the embryos of seeds of the mutant are normal, the endosperm is imperfectly developed; this results in extremely poor germination.

Crosses of the pigmented mutant with *cAR*, *CAR* and *CA*^{p}*r* showed one, two and four factor differences, respectively. On the basis of this evidence, it is concluded that the induced change is at a newly identified locus, *Pi*, and is recessive to the normal state; the action of *pi* is dependent on the presence of two doses of *R*; *p*$_i$ is epistatic over *C*; *P*$_i$ or at least one dose of r is essential for the normal functioning of *C*; and in the absence of *C*-controlled anthocyanin, *A* and *A*^{p}, the two alleles of *A* tested, have no recognizable action.

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Chromosome Aberrations and Growth Hormone

(*Continued from page* 52)

tom could be helped with growth hormone. The patient was not helped to the degree that pituitary dwarfs (whose small stature is due to underfunctioning of the pituitary gland) are helped, however; the hormone, as used here, almost quadruples the growth rate of pituitary dwarfs treated by the group.

The findings have raised questions of a fundamental nature. If one symptom of a chromosomal abnormality can be alleviated by one hormone, can other symptoms be lightened by still other hormones?

Dr. N. R. Moudgal in the Berkeley group, using a delicate method of measuring growth hormone in circulating blood, has found that acromegalics have detectable growth hormone in their blood and the hormone cannot be found in the circulation of normal people.

The system of measuring pituitary hormones —which are large protein molecules—suggests a theoretical method of treating conditions caused by hormone overproduction.

This is how blood levels of growth hormone are measured: 1) Purified human growth hormone is injected into rabbits; 2) the rabbits produce antibodies against the hormone; 3) the antibody-containing part of the rabbit blood is added to a test tube of the blood to be tested; 4) the rabbit's antibodies precipitate the growth hormone in the human blood, and 5) the size of the test tube precipitate indicates the amount of hormone in the blood.

The success of this system as a measuring device has raised the question as to whether antibodies could be used to knock out excess protein hormones in patients suffering from overactive pituitaries. Would injections of antibody-loaded rabbit serum destroy the excess hormones?

The observations can be stretched even as far as a theoretical treatment for some cancers.

If pituitary hormones are involved in the induction and maintenance of growth of some cancers, would rabbit antisera against the hormones knock out the hormones and arrest the cancer growth?

At this time the question remains purely academic because animal experiments to this end have not yet been done—and even had they been done and found successful—there is not enough growth hormone available for trials on patients.

Dr. Li's and several other groups for many years have been trying to achieve the total laboratory synthesis of growth hormone; but, despite considerable progress in purifying the substance, it may be a long time until total synthesis is achieved and widespread clinical use made possible.

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